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CONTENTS

INVESTIGATION OF PRIMARY AND SECONDARY METABOLITES IN A CHEMICAL STUDY OF *CORTINARIUS ARMILLATUS* (*CORTINARIACEAE, TELAMONIA*)

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ABSTRACT. - The fruit-bodies of *Cortinarius armillatus* were investigated for polyols, sugars, phenolic acids, alkaloids and fungal toxins using thin-layer chromatography methods. Arabitol, mannitol, trehalose, fructose, 4-hydroxybenzoic acid, choline and cortinarine A were detected from aqueous and methanolic extracts of the mushroom. The fungal toxins, α-amanitin, orellanine, muscarine, muscimol and bufotenine were not observed. These chemical investigations and acute toxicity studies in mice supported the non-toxicity of *C. armillatus*.

RÉSUMÉ. - Une étude chimique des métabolites primaires et secondaires a été réalisée sur *Cortinarius armillatus* par chromatographie sur couche mince. L'arabitol, le mannitol, le tréhalose, le fructose, l'acide parahydroxybenzoïque, la choline et la cortinarine A ont été mis en évidence dans les extraits méthanoliques et aqueux de *C. armillatus*. Les toxines fongiques, α-amanitine, orellanine, muscarine, muscimol et bufotenine n'ont pas été observées dans ces mêmes extraits. La non-toxicité de *C. armillatus* est confirmée par des études chimiques et pharmacologiques sur la souris.

Key-words: *Cortinarius armillatus*; cortinarine A; arabitol; mannitol; 4-hydroxybenzoic acid; choline.

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INTRODUCTION

Cortinarius armillatus (Fr. : Fr.) Fr. is a large, reddish-brown edible-like mushroom with a clubshaped stalk and irregular reddish bands, single to several, in deciduous woods (Cetto, 1978; Marchand, 1983). To the best of our knowledge, no data exists as to the chemical constituents of *C. armillatus* except pigment composition (Besl *et al.*, 1978). In this work, an examination of polyols, sugars, phenolic acids, alkaloids and other fungal secondary metabolites was undertaken using mono- and two-dimensional thin-layer chromatography (TLC) spraying with selective reagents. On the other hand, acute toxicity studies were also carried out and had never been previously reported.

MATERIAL AND METHODS

1. Material

C. armillatus was collected at Regensburg in Bavaria in october 1993 and preserved by drying after morphological identification from fresh material.

2. Methods

Preparation of extracts

20 g of *C. armillatus* fruit-bodies finely powdered were extracted with methanol (100 ml x 5) by sonication (Ultra Sonik 300 NEY) at room temperature for 1 h.

The methanolic combined extracts were filtered on Durieux filter paper n°113 and evaporated to dryness in a rotary vacuum evaporator at 35-40°C. Aliquots of the residue were added with methanol for thin-layer chromatography analyses (polyols, sugars, alkaloids, fungal toxins) or resuspended in carboxymethyl cellulose for pharmacological investigations.

The powder of mushroom was then re-extracted with water (100 ml x 3) by sonication in the same conditions as previously described. After filtration, the combined aqueous solutions were freeze-dried. The residue was added with water for TLC analyses (polyols, sugars, phenolic acids).

Qualitative determination of polyols and sugars

The methanolic and aqueous extracts were analyzed on silica 60 F254 plates (Merck, ref. 5735) according to Andary *et al.* (1979) in the following solvent systems (V/V): acetone-water (9:1) and methylene chlorure-acetic acid-methanol-water (50:5:20:5) in the same dimension. The TLC profiles of the extracts were compared with 0.015 % polyol and 0.1 % sugar standard solutions (W/V) in ethanol-water (98:2, V/V). Rf values were: arabitol, Rf = 0.48; mannitol, Rf = 0.34; fructose, Rf = 0.44; glucose, Rf = 0.40 ; galactose, Rf = 0.36 and trehalose, Rf = 0.16.

Glucose, fructose and galactose were distinguished using TLC on cellulose plates (Merck, ref. 5577) developed in *n*-butanol-ethanol-water (4:1:2.2, V/V) up to 4 cm solvent front, and sprayed with 0.2% naphthoresorcinol in ethanol (W/V) with 5% sulfuric acid (Rapior *et al.*, 1990). The three hexoses were detected as follows: fructose,

Rf = 0.36, dark fuchsia; glucose, Rf = 0.32, turquoise blue and galactose, Rf = 0.30, pale blue. The polyols and the disaccharide were not revealed by this method.

Qualitative estimation of phenolic acids

A 100 mg aliquot of the water extract was added with water. The solution was adjusted to pH 3 with 10% acetic acid and partitioned with diethyl ether. The organic phase was evaporated to dryness and the residue added with 50% methanol (2 ml). The hydromethanolic solution was analyzed by two-dimensional TLC on cellulose plates (Merck, ref. 5577) developed in 2% acetic aqueous acid (AA) and toluene-acetic acid-water (60:28:1.2, TAW, V/V) (Rapior *et al.*, 1990). The chromatograms were sprayed with 4-nitroaniline reagent (Stahl, 1969). The TLC profile of the extract was compared with 0.1% phenolic acid standard solutions (W/V) in methanol.

Rf values were: 4-hydroxyphenylacetic acid (Rf = 0.81 in AA, Rf = 0.40 in TAW, mauve); 4-hydroxybenzoic acid (Rf = 0.56 in AA, Rf = 0.46 in TAW, pink); 4-hydroxycinnamic acid or p-coumaric acid (Rf = 0.41 in AA, Rf = 0.49 in TAW, blue-grey); 3,4-dihydroxyphenylacetic acid (Rf = 0.75 in AA, Rf = 0.11 in TAW, purple); 3,4-dihydroxybenzoic acid or protocatechuic acid (Rf = 0.63 in AA, Rf = 0.16 in TAW, mauve-grey) and 4-hydroxy 3-methoxybenzoic acid or vanillic acid (Rf = 0.50 in AA, Rf = 0.67 in TAW, purple).

Qualitative detection of alkaloids, quaternary ammonium compounds and α-amanitin

The methanolic extract was chromatographed on cellulose plates (Merck, ref. 5577) using methanol-water-acetic acid (90:5:5, V/V) as mobile phase. TLC plates were sprayed with Dragendorff's reagent modified according to Bregoff-Delwiche (Stahl, 1969) for alkaloids and quaternary nitrogen compounds and, with sulfanilic acid diazotised for α-amanitin, bufotenine and muscimol (Andary *et al.*, 1977).

Concentrations of standard solutions (W/V) and Rf values were: betaïne (0.2% in methanol, Rf = 0.56, orange); choline chloride (0.25 % in methanol-water (1:1), Rf = 0.70, brown); muscarine (0.01% in methanol, Rf = 0.82, orange pale); α-amanitin (0.1% in methanol, Rf = 0.65, brownish-pink); bufotenine hydrogenoxalate (0.1% in methanol-water (1:1), Rf = 0.71, pink) and muscimol (0.5% in methanol, Rf = 0.52, pink orange).

Qualitative estimation of other fungal metabolites: cortinarine A and orellanine

The methanolic extract of *C. armillatus* was analyzed for cortinarine A by TLC on silica 60 F254 support (Merck, ref. 5735) in the following monodimensional solvent systems (V/V) : *n*-butanol-acetic acid-water (4:1:1, BAW) and cyclohexane-ethyl acetate (3:1, CHEA) (Caddy *et al.*, 1982) and compared with a methanolic extract of *Cortinarius orellanus* Fr. Cortinarine A appeared as a blue fluorescent spot after exposure to UV light at 254 nm.

Orellanine was detected from the methanolic extract of *C. armillatus* by TLC on cellulose plates (Merck, ref. 5716) in *n*-butanol-hydrochloric acid-chloroform-water (40:20:15:3.8, BCCE, V/V) as mobile phase and compared with a 0.002% orellanine standard solution (W/V) in methanol-water (1:1, V/V) (Rapior *et al.*, 1988). Orellanine

appeared in the form of a dark spot which, after exposure for 1-3 minutes to UV light at 366 nm, produced a bluish-white fluorescence characteristic of orelline, the photodecomposition product of orellanine.

Rf values were: cortinarine A , Rf = 0.86 in BAW and Rf = 0.50 in CHEA; orellanine, Rf = 0.60 in BCCE.

3. Acute toxicity studies

Adult, male and female SWISS mice from 4 to 5 weeks old weighing between 24-26 g and 15-20 g respectively, procured from the Department of Pharmacology (Faculty of Pharmacy, Montpellier) were used for these experiments. The animals were housed 3 male and 5 female per cage (25 x 45 x 15 cm). The room temperature was 22°C and humidity 60 ± 10 %. Artificial light was the only source of light and the animals were set on a 12 hour light/dark cycle. They had free access to commercial pelleted diet (UAR A04) and tap water. The animals had been fasted 18 hours before they were intraperitoneally (i.p.) injected with suspensions of the methanolic extract residue from *C. armillatus* in 3% aqueous carboxymethyl cellulose. The following single doses were given to 6 male (2000, 1000, 500 and 250 mg/kg) and 8 female (2000 mg/kg). All suspensions were prepared in such a manner that 10 ml was given per kg of body weight. The animals were weighed on day 1, 5, 10 and 15, and were sacrified on day 15 for anatomical observations of thoracic and digestive organs.

RESULTS AND DISCUSSION

1. Polyol and sugar contents

Polyols were generally considered independent of other primary metabolites but they frequently coexist in mushroom extracts with free sugars, which are related carbohydrate compounds. Using the methods by Andary *et al.* (1979) and Rapior *et al.* (1990), arabitol and mannitol were detected from the aqueous and methanolic extracts of *C. armillatus*. Both extracts contained trehalose as also reported for *Cortinarius*, subgenus *Leprocybe*, section *Orellani* (Rapior *et al.*, 1990) and *Boletus* (Benedict and Tyler, 1968). Fructose was present only in the methanolic extract of *C. armillatus*.

2. Phenolic acid content

Chromatographic examination of the water extract from *C. armillatus* demonstrated only presence of 4-hydroxybenzoic acid. It was observed as the major phenolic acid constituent from seven *Cortinarius* species of section *Orellani* (Rapior *et al.*, 1990).

3. Alkaloid and nitrogen compound contents

C. armillatus contained choline. As reported for *Entoloma* (Maki *et al.*, 1985), presence of choline proved to be of no significance chemotaxinomically. Betaine was not detected by our method.

4. Other fungal metabolite content

Cortinarine A was detected from the methanolic extract of *C. armillatus*. Similar result was obtained on analysis of 59 *Cortinarius* species from the seven subgenera of the *Cortinarius* genus (Tebbett and Caddy, 1983).

On the other hand, the fungal toxins, α-amanitin, bufotenine, muscarine, muscimol and orellanine were not identified from the fruit-bodies of *C. armillatus*. For this reason it is suggested that *C. armillatus* fruit-bodies should now be considered to be potentially non toxic.

5. Toxicity studies

All mice survived the observation period of 15 days. Male and female mice given single i.p. doses of the methanolic extract from *C. armillatus* had no toxic symptoms and no organic pathology up to 2000 mg/kg, two weeks after dosing. This was confirmed by the evolution of the animal weight at different doses for the same period of time. Autopsy of animals sacrified on day 15, revealed no digestive and pulmonary changes.

CONCLUSION

TLC screening of the methanolic and aqueous extracts of *C. armillatus* revealed the presence of polyols (arabitol, mannitol), sugars (trehalose, fructose), phenolic acids (4-hydroxybenzoic acid), nitrogen compounds (choline) and other fungal metabolites (cortinarine A). Mice given single intraperitoneal doses (from 2000 to 250 mg/kg) of a suspension from the methanolic extract of *C. armillatus* had no symptoms and no organic pathology two weeks after dosing. These chemical studies and pharmacological investigations supported the non-toxicity of *C. armillatus*.

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OCCURRENCE OF MELANIN IN BRIGHT-SPORED MYXOMYCETES

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ABSTRACT - In a study of spore wall pigments of eleven species of the Orders Liceales and Trichiales - taxa which are traditionally separated from the dark-spored Orders Physarales and Stemonitales by their variously coloured spores - it was found that even in these bright-spored Orders, melanin was present in the spore walls of all the species. In addition, there were several organic-solvent-extractible pigments, usually in low amounts.

RÉSUMÉ - Ce travail a eu pour but l'étude des pigments pariétaux de 4 espèces appartenant aux ordres des Liceales et des Trichiales, taxons habituellement isolés des Physarales et Stemonitales, ordres à spores pigmentées, en raison de leurs coloration variable. Il a été démontré que les parois des spores de ces 4 espèces, à pigmentation claire, contenait de la mélanine. En plus, la présence en faible quantité de pigments extractibles par les solvants organiques a pu être démontrée.

KEY-WORDS - Myxomycetes, spore pigments, melanin, Liceales, Trichiales.

INTRODUCTION

The occurrence of melanin as the only pigment in the spore walls of Myxomycetes belonging to the dark-spored orders Physarales and Stemonitales, has been reported (Loganathan *et al.*, 1989). We studied the spore wall pigments of some species belonging to the Liceales and Trichiales - traditionally the bright-spored group - on which relatively new studies have been made (Liaane-Jenson, 1965; Blackwell & Busard, 1978; Czeczuga, 1980; Steglich *et al.*, 1980; Kopanski *et al.*, 1982, 1987).

MATERIALS AND METHODS

Materials

For extraction of pigments, spores are required in large quantities. The Liceales and Trichiales generally favour temperate climates. Specimens were collected by the first author in the foothills of the Himalayan mountains in the state of Himachal Pradesh (H.P.), at 31°15' to 31° 45' N latitude, 77° 25' to 77° 60' longitude, at elevations of 2300 to 3000 m, in September 1989. They were identified after Lister (1911), Martin & Axelopoulos (1969) and Emoto (1977). Twelve species were selected for study. Spore colours were determined with reference to Rayner (1970). Details of

the material are listed in Table I. One species of the Stemonitales was included for comparison.

Table I - Material used

Order and specific name	Herbarium N°	Source
LICEALES		
**Lycogala epidendrum* (L.) Fries	MUBL/IK/FC/185	Fresh collection from H.P. (a)
Reticularia lycoperdon Bull.	HPUB/12750	From the collection of Dr TNL (b)
Cribraria atrofusca Martin & Lovejoy	MUBL/IK/FC/194	Fresh collection from H.P.
TRICHIALES		
Arcyria ferruginea Fuckel	HPUB/12604	From the collection of Dr TNL
A. nigella Emoto	MUBL/IK/FC/182	Fresh collection from H.P.
**A. occidentalis* (Macbr.) G. Lister	MUBL/IK/FC/176	"
A. stipata (Schw.) A. Lister	MUBL/IK/FC/178	"
**Trichia decipiens* (Pers) Macbr.	MUBL/IK/FC/173	"
T. favoginea Schum.	MUBL/IK/FC/170	"
T. varia (Pers.) Pers.	MUBL/IK/FC/172	"
**Hemitrichia serpula* (Scop.) Rost.	MUBL 2882	Earlier collection from Coorg, Karnataka
STEMONITALES		
Stemonitis fusca Roth	MUBL/IK/FC/196	Fresh collection from H.P.

* Non-melanin pigments also studied
(a) Himachal Pradesh
(b) Prof. Dr T.N. Lakhanpal, Biosciences Department, H.P. University, Shimla.

Extraction of pigments

Whole spores were used for all extractions. These were separated by manually shaking dry sporangia in small closed boxes until the sporangial structures separated from spores, rolled up into a ball and could be removed *en masse*. The spores were collected in small test tubes, sealed and stored at 4°C until needed. Non-melanin pigments were extracted only from four species (marked with an asterisk in Table I), the amount of spores collected from the others being insufficient for two types of extraction.

Melanin was extracted by standard methods (Thomas, 1955) from pre-weighed spores with 1 M KOH, and purified as detailed earlier (Loganathan *et al.*, 1989). After drying, the weight of this purified pigment was determined. Non-melanin pigments were extracted by boiling about 10 mg of spores in 5 ml of ethanol in a closed tube for 1 h. After cooling, the material was centrifuged at 2000 rpm and the supernatant collected. The process was repeated until no more pigment could be extracted. The extracts were pooled and concentrated over a water bath. As very little pigment could be extracted by this method, the method described by Steglich *et al.*, (1980) was tried with *Trichia decipiens*, of which there was a plentiful supply. Following ethyl acetate,

several other solvents such as methanol, acetone, diethyl ether and dimethyl sulfoxide were tried in succession, but only part of the pigment could be extracted and the spores still remained coloured.

Analysis

Melanin was subjected to the various physical and chemical tests as detailed in Loganathan *et al.* (1989).

Non-melanin pigments were separated by thin-layer chromatography on silica gel-G (Merck). The plates were developed with Toluene:Formic acid-ethyl acetate:Formic acid (10:10:3) (Kopanski *et al.*, 1982). The spots thus separated, however, were too faint in most cases for further elution and testing. The chromatographs of *T. decipiens* were developed in methanol, after initial testing with the above solvent system and a few others. A single spot which developed from each extract, was eluted and read in a Beckmann DU-40 spectrophotometer at the range of 200 to 500 nm.

RESULTS

Melanin

A brown pigment was extracted with 1 M KOH from all the 12 species, and a range of tests (Loganathan *et al.*, 1989) showed the pigment in all the species to be melanin.

The ultra-violet spectra of the melanins from the 12 species showed a fairly uniform pattern (Fig. 1). The Liceales showed the highest absorption at 220 to 223 nm, as most clearly exemplified in *Cribraria atrofusca*. In the Trichiales the peaks were also seen at 221-223 nm, and were sharply defined in all species. A hump at 280 to 310 nm, and were sharply defined in all species. A hump at 280 to 310 nm was seen in most of the species in both the orders, but it was not very prominent except in the Trichias and in *Hemitrichia serpula*. In *Stemonitis fusca*, the absorption peak was seen at 222 nm.

The infra-red spectra of the melanins of the 12 species showed variations (Fig. 2). The prominent characteristics were:

a. Sharp absorption peaks at 3.3 to 3.4 μm (2860 & 2930 cm^{-1})
b. A broad band at 4.3 to 5.0 μm (2000-2400 cm^{-1})
c. An absorption peak at 5.7 to 6.0 μm (1715 cm^{-1}).

In addition to these, absorption at 4.1-4.3 μm (2300-2500 cm^{-1}) was uniformly seen in all the 12 species, but this was never prominent. Based on the presence or absence/ prominent or diffuse nature of these features, the 12 species could be broadly separated into four groups, but this grouping had no bearing on their taxonomic status (Table II).

The percentage of melanin in relation to the fresh weight of the spores was determined only in two species of the Liceales, amounting to 7-10%, and four species of the Trichiales amounting to 4.5% in *Trichia* and 3% in *Arcyria* (Table III). The dry weight of the spores was determined only in *T. decipiens* where a separate extracion gave 6.8% melanin.

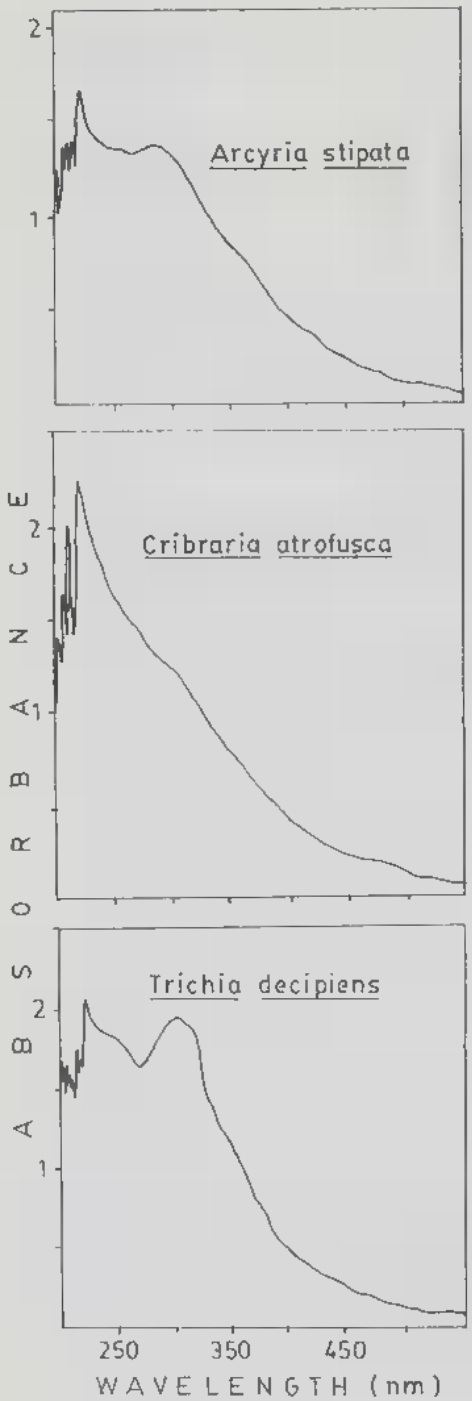

Fig. 1 - Ultraviolet spectra of melanin from three representative species of the Trichiales and Liceales, showing characteristic absorption patterns.

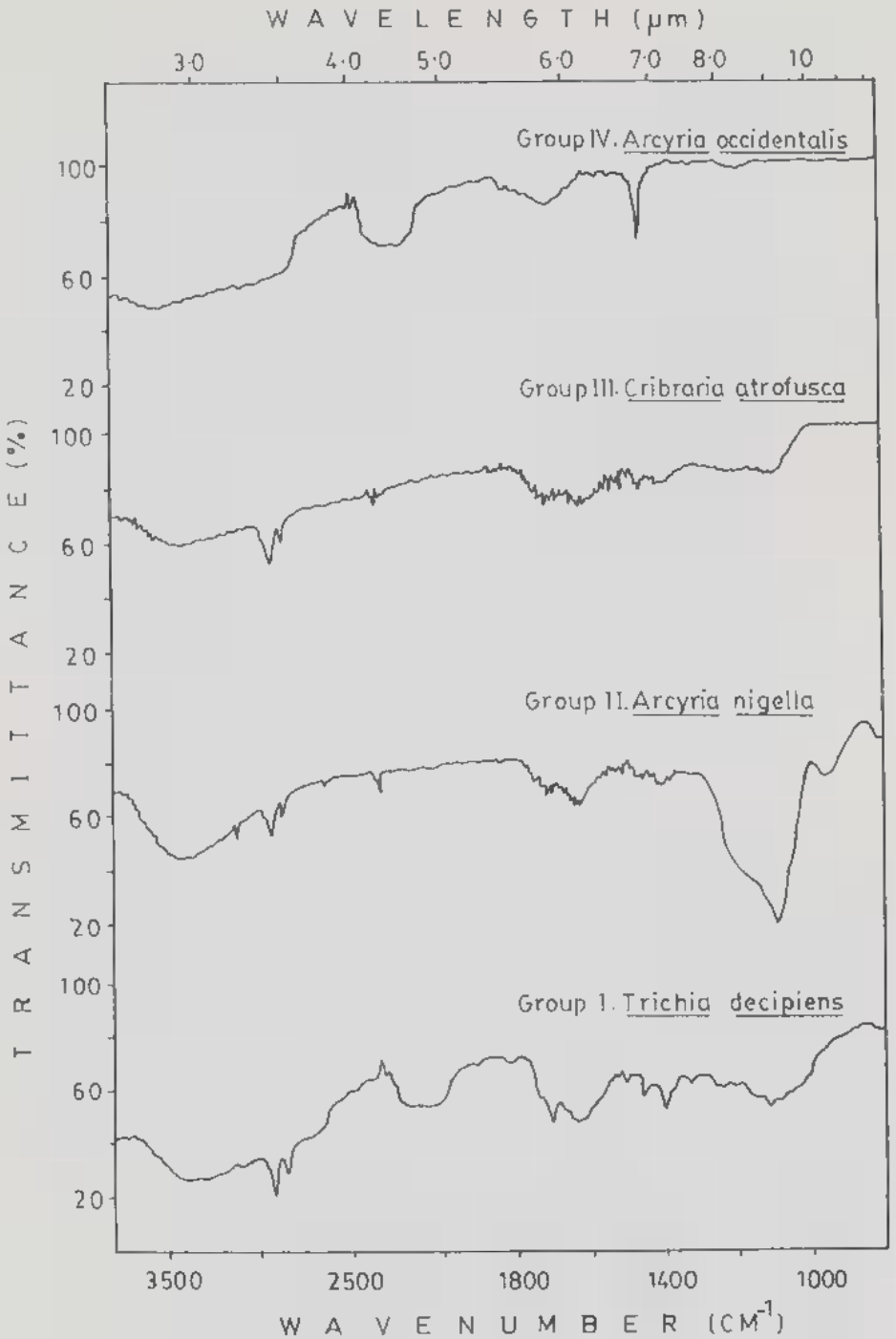

Fig. 2 - Infra-red spectra of melanin from the Trichiales and Liceales, representing the four groups described in the text.

Table II - Infra red patterns of melanins of Liceales, Trichiales and *Stemonitis*.

Grouping	species	Features			
		a Peaks at 3.3-3.4 µm	b Trough at 4.3-5.0 µm	c Peaks at 5.7-6.0 µm	Remarks
I	*Lycogala epidendrum*	+	+	+	
	Trichia decipiens	+	+	+	
II	*Arcyria stipata*	+	-	+	
	A. nigella	+	-	+	Additional peak at 9µm
	Trichia favoginea	+	-	+	
	T. varia	+	-	+	
	Stemonitis fusca	+	-	+	
III	*Cribraria atrofusca*	+	-	-	
	Reticularia lobata	+	-	-	
	Arcyria ferruginea	+	-	-	
IV	*Arcyria occidentalis*	-	+	Diffuse	Additional sharp peak at 7 µm
	Hemitrichia serpula	-	+	Diffuse	

Table III - Summary of spore wall pigments in the twelve species studied.

Order and Species	Spore colour* (Nearest shade: Rayner)		Melanin	Percentage (W/W) (Fresh wt)	Number of other pigments in ethanol extract
	Term	Notation			
LICEALES					
Lycogala epidendrum	Buff	19''f	+	10.0	6
Reticularia lobata	Dark brick	7''k	+	-	-
Cribaria atrofusca	Umber	13 m	+	7.0	-
TRICHIALES			+		
Arcyria ferruginea	Bay	5 k	+	-	-
A. nigella	Sepia	13''k	+	3.0	-
A. occidentalis	Umber	13 m	+	-	4
A. stipata	Fawn	11''	+	3.0	-
Trichia decipiens	Umber	13 m	+	4.5	3
T. favoginea	Amber	19'	+	4.5	-
T. varia	Ochreous	13'b	+	-	-
Hemitrichia serpula	Umber	13 m	+	-	5
STEMONITALES					
Stemonitis herbatica	Chesnut	5'm	+	-	None

+ : present; - : not done

* In a few cases, spores were somewhat faded at the time of colour determination.

Non-melanin pigments

The solvents for pigment extraction and for developing the chromatographs were selected after preliminary trials with several solvent combinations. Ethanolic extracts were analysed from *Lycogala epidendrum, Trichia decipiens, Arcyria occidentalis* and *Hemitrichia serpula*. For the other species, the spores were used up for melanin extraction. Boiling with ethanol did not give complete extraction, as the spores still remained coloured. The pigments of *T. decipiens* were extracted with a series of solvents but the spores still remained coloured. Chromatographic separation of the extracts revealed six fractions in *Lycogala*, three in *Trichia*, four in *Arcyria* and five in *Hemitrichia*, with Rf values ranging from 0.2 to 0.9. The UV absorption of the pigments extracted from *Trichia* in different solvents is presented in Table IV. When the dried pigments were pooled together, they formed 7.5% of the dry weight of spores.

Table IV - UV absorbance of pigments extracted from *Trichia decipiens*.

Solvent	Absorbance at (wave length in nm)				
	230	250	270	290	300
Ethyl acetate	-	+	-	-	-
Dimethyl sulfoxide	-	-	+	-	-
Diethyl ether	+	-	-	+	-
Ethanol	-	+	-	-	+
Water	+	-	-	-	-

DISCUSSION

Melanin

Although melanin has previously been reported in a Liceaceous species (Loganathan *et al.*, 1989), its occurrence in all the other species including the bright-spored Arcyrias and Trichias, was a surprise. Melanin forms 3 to 10 per cent of the fresh weight, or up to 7 per cent of the dry weight of whole spores. In the dark-spored orders where melanin is the only pigment, it constitutes 7 to 15 per cent of the dry weight of separated spore walls (McCormick *et al.*, 1970; Chapman *et al.*, 1983; Paramasivan, 1990). From our results in *Trichia decipiens*, there is an indication that the melanin and non melanin pigments may occur in equal amounts.

Apparently, in the bright-spored species, melanin is masked by other pigments. Sporangia of Trichias, still in the process of development, are of a shiny black colour and if crushed, would exude the still semi-liquid contents as a purplish-black fluid. The characteristic brownish yellow colour of the mature sporangia develops much later. The non-melanin pigments could be extracted from spores before, but not after the extraction of melanin, apparently being destroyed by the drastic procedures of melanin extraction. It seems reasonable to presume that the bright pigments appear after the completion of melanin synthesis, and that they occur at the surface.

The UV spectra of melanin from both the Liceales and Trichiales showed a fair degree of uniformity, with peaks at 221-223 nm. In this respect they were similar to the melanins of the Stemonitales (Loganathan *et al.*, 1989). Of the hump at 280-310 nm, seen in several species, there had been only a faint suggestion in the Physarales and Stemonitales.

The i.r. spectra showed similarities to and differences from those of the Physarales and the Stemonitales. The absorption around 3 μm (2860 & 2930 cm^{-1}), which was seen in all but two species, had been seen in both the dark-spored orders, although it was not equally prominent in all the species. The broad absorption at 4-5 μm, seen in *Lycogala epidendrum*, *Trichia decipiens*, *Arcyria occidentalis* and *Hemitrichia serpula*, and as low bands in all the species, had been seen as well-defined peaks in all the Physarales and Stemonitales (Loganathan *et al.*, 1989). The peak at 1715 cm^{-1}, clearly seen in *Lycogala epidendrum* and *Trichia decipiens* and in a diffuse way in some Arcyrias and Trichias, had been seen in all the members of Physarales and the Stemonitales.

The absorption at 9 μm (1000-1300 cm^{-1}), seen in *Arcyria nigella*, is similar to that described by Rast *et al.* (1981) as being characteristic of the GDHB melanin of *Agaricus bisporus*, and we have reported it earlier in *Reticularia lycoperdon* (Loganathan *et al.*, 1989).

Non melanin pigments

The separation into 3 to 6 fractions in our study is thus in accordance with earlier findings. Blackwell & Busard (1978) reported 3 to 6 fractions in the ethanolic extracts of some Trichiaceous species, with considerable intra- and interspecific variation. Steglich and his associates obtained several fractions from *Arcyria denudata* (Steglich *et al.*, 1980), *Trichia floriformis* and *Metatrichia vesparium* (Kopanski *et al.*, 1982, 1987). Czeczuga (1980) reported several carotenoid pigments from each of eight species of Myxomycetes, which represented all the four major orders. In the earlier studies, however, whole sporangia were used for pigment extraction and not the spores alone. The chemical nature of these pigments has been definitely established only in a few cases, through the studies of Steglich and his associates (Steglich *et al.*, 1980; Kopanski *et al.*, 1982, 1987).

Considering that the yellow and brown pigments of the Liceales and Trichiales become non-extractible after melanin extraction, and are not completely extractible even before melanin extraction, it is possible that they occur closely bound to the melanin molecule. Verification of such a hypothesis, however, has to await further study.

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ALGUNOS *AGARICALES* DE LAS PLAYAS DE ESPAÑA PENINSULAR

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RESUMEN - Se citan o describen catorce táxones de *Agaricales s. l.* que fructifican en arenas marítimas. En general son especies poco conocidas y apenas citadas en la Península Ibérica. Desde el punto de vista corológico son de interés para la distribución de la micoflora europea. Se aportan microfotografías al microscopio óptico y electrónico de barrido (MEB) de sus características más importantes. Se propone la nueva combinación *Calocybe chrysenteron* var. *juncicola* (R. Heim) G. Moreno y la nueva variedad *Melanoleuca polioleuca* var. *confusa* G. Moreno.

ABSTRACT - Fourteen taxa of Agaricales s. l. growing in maritime sands are recorded or described upon. Altogether they are poorly known and most of them have only been recorded a few times in the Iberian Peninsula. From a chorological point of view, they are interesting for the distribution of the European mycoflora. Microphotographs, made under ■ the optical and scannning electronic microscope (SEM) of their most striking features are added. One new combination, Calocybe chrysenteron var. juncicola (R. Heim) G. Moreno and one new variety *Melanoleuca polioleuca* var. *confusa* G. Moreno are proposed.

RÉSUMÉ - Nous citons ou décrivons quatorze espèces d'*Agaricales s. l.* qui fructifient sur les sables maritimes. Généralement ce sont des espèces peu connues et à peine citées dans la Péninsule Ibérique. Du point de vue corologique elles sont intéressantes pour la distribution de la mycoflore européenne. Nous adjoignons des microphotographies au microscope optique et électronique à balayage de leurs caractéristiques les plus importantes. Nous proposons la nouvelle combinaison *Calocybe chrysenteron* var. *juncicola* (R. Heim) G. Moreno et la nouvelle variété *Melanoleuca polioleuca* var. *confusa* G. Moreno.

KEY WORDS: Taxonomy, Chorology, Psammophylous fungi, *Agaricales s. l.*, Spain.

INTRODUCCION

Con motivo de la asistencia por parte de uno de nosotros (GM) a las exposiciones micológicas de Santander en los años 1992 y 1993, tuvimos ocasión de recorrer las excelentes playas de Liencres, y recoger abundante material de *Agaricales s. lato*. Algunas de estas especies, junto con otras recogidas de otros lugares pero de similar ecología, son descritas en este trabajo. Destacamos su hábitat característico psamófilo o arenícola, y su rareza en la Península Ibérica.

El material se conserva en el herbario AH (Dpto. Biología Vegetal, Universidad de Alcalá de Henares).

Las microfotografías han sido obtenidas en un microscopio Nikon modelo Optiphot, con sistema automático de fotografía incorporado y contraste de fases. Hemos utilizado hidróxido amónico 10%, rojo congo amoniacal y reactivo de Melzer principalmente.

Agaricus devoniensis P. D. Orton, *Trans. British Mycol. Soc.* 43: 177. 1960

Poco frecuente en dunas estabilizadas con *Ammophila arenaria*, Ribera de Cabanes (Castellón), *leg.* J. Ayllón & G. Moreno, 6-XII-1993, AH 16624; En dunas fijadas con *Juniperus phoenicia* subsp. turbinata, Punta Entinas (Almería), *leg.* V. González, C. Illana & A. Altés, 3-XII-1993, AH 16622; *Ibidem, leg.* G. Moreno, C. Illana & A. Altés, 15-II-1994, AH 16623.

Observaciones: *Agaricus devoniensis* se caracteriza por su sombrero blanquecino con máculas amarillentas más o menos abundantes y persistentes en herbario, por su porte pequeño a mediano (2-7 cm de diám.) y por el anillo, situado en la zona media a basal del pie que rompe con facilidad, originando un segundo anillo inferior característico.

Nuestras recolecciones coinciden con la descripción e iconografía de Cappelli (1984).

Calocybe chrysenteron* var. *juncicola (R. Heim) G. Moreno, *comb. nov.* (Fig. 1)
≡*Tricholoma chrysenteron* var. *juncicola* R. Heim, Treb. Mus. Ci. Nat. Barcelona 15 (3): 101. 1934.

Gregario cerca de *Juncus maritimus* y *Plantago crassifolia*, Ribera de Cabanes (Castellón), *leg.* A. Burguete, 17-XI-1991, AH 16514.

Sombrero de 1-1,5 cm. de diám., de color amarillo uniforme con tonos amarillos crémeos en el centro, seco o débilmente viscoso. Margen decurvado a plano. Láminas adnatas con lamélulas, poco apretadas, concoloras al sombrero inclusive la arista. Pie cilíndrico, de 2,5-3,5 x 0,3-0,4 cm, concoloro al sombrero, a veces con una pruina blanquecina en ejemplares jóvenes. Carne de sabor dulzaino y olor farinoso típico. Color al corte no observado.

Pileipellis formada por hifas cilíndricas estrechas, 2-3 µm de diám., fibuladas, entrelazadas. Basidios tetraspóricos, de 22-25 x 6-7,5 µm, claviformes. Esporas elipsoidales, de 5-6 x 3-3,5 (4) µm, hialinas, lisas, no amiloides ni dextrinoides. Cistidios faciales y marginales no observados. Al realizar la preparación en KOH 10% se observa una tonalidad violácea en la solución

Observaciones: *Calocybe chrysenteron* var. *juncicola*, pertenece al complejo de *C. chrysenteron* caracterizado por la pileipellis filamentosa y esporas pequeñas. *C. chrysenteron* es una especie que suele fructificar en humus de coníferas y posee esporas más pequeñas (2,5-3,5 x 2-3 µm) y la carne es de color amarillento, (Kühner & Romagnesi, 1974 ; Breitenbach & Kränzlin, 1991)

Heim (1934), describe una variedad nueva: *Tricholoma chrysenteron* var. *juncicola*, para una recolección muy parecida por las coloraciones amarillentas del

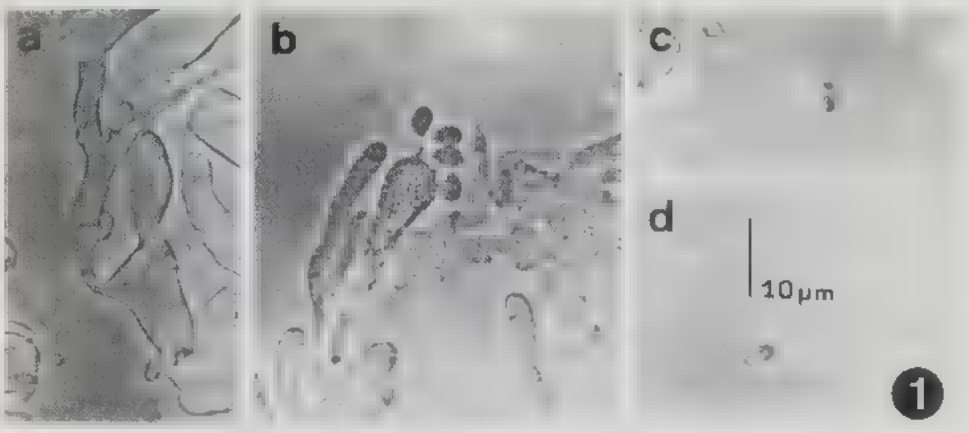


Fig 1. - *Calocybe chrysenteron* var. *juncicola* (AH 16514); a: fíbula; b: basidio tetraspórico; c-d: esporas.

cuerpo fructífero a *Calocybe chrysenteron,* y la diferencia después de consultar con Maire, por el hábitat higrófilo, (en restos de *Juncus acutus*), carne al corte de color blanquecino y esporas de 5,5 x 3-3,5 µm.

Posteriormente esta variedad ha sido interpretada como especie diferente, por Kühner & Romagnesi (1974) y Singer (1977).

El material recogido en Castellón tiene un hábitat semejante al indicado por Heim (loc. cit.). Nuestro colaborador (A. Burguete) nos envió el material (tres carpóforos maduros) y una excelente diapositiva, pero no precisó el color de la carne, la cual no hemos podido observar en el material desecado, por lo que no hemos podido verificar este último carácter; sin embargo, el resto de las características, incluyendo las dimensiones esporales, coinciden bien con la descripción original de Heim. Creemos, a pesar de las limitaciones que conlleva tener una sola recolección, que esta especie es muy próxima a *Calocybe chrysenteron,* y bien pudiera tratarse de una variedad que crece en áreas sin vegetación arbórea, en humedales (albuferas) entre plantas higrófilas como *Juncaceae*, con esporas ligeramente mayores que el tipo. El color de la carne al corte convendría controlarlo en sucesivas recolecciones para precisar su constancia. Estas razones nos hacen tratarlo en este trabajo de forma semejante a la de Heim (loc. cit.) y realizar la combinación válida como variedad de *C. chrysenteron.*

Conocybe dunensis T. J. Wallace *in* P. D. Orton, Trans British Mycol. Soc. 43:192. 1960. (Fig. 2)

Abundante, pero localizado en dunas fijadas, Liencres (Santander), *leg.* G. Moreno, 19-X-1993, AH 16515.

Píleo de 2-3 cm de diám., convexo-campanulado, mamelonado, liso, de color pardo dátil a ocráceo. Margen entero ▪ ligeramente decurvado. Láminas adnatas, apretadas, de color ocráceo y con lamélulas. Pie de 2-9 x 0,1-0,2 cm, cilíndrico, recto,

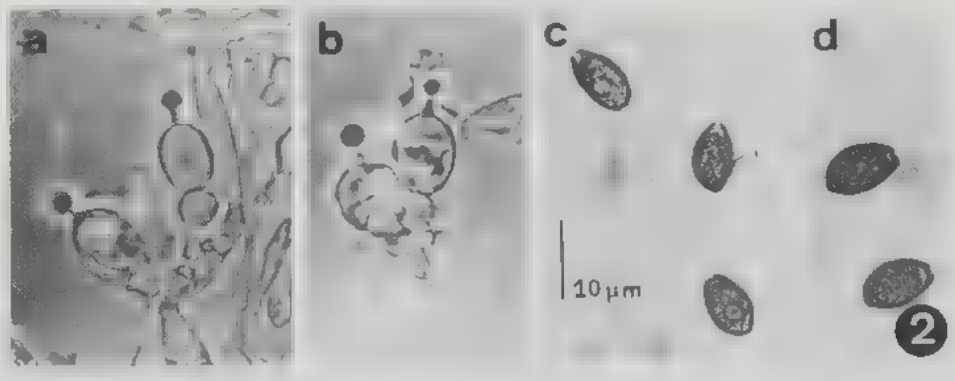


Fig. 2. - *Conocybe dunensis* (AH 16515); a-b: cistidios; c-d: esporas.

radicante, enterrado en la arena hasta los 2/3 de su longitud, de color crema pálido, con pruina blanquecina copiosa en su parte superior. Olor y sabor no apreciables.

Pileipellis himeniforme, formada por células claviformes de 40-65 x 17-20 µm. Esporas elipsoidales, de 12-14 x (5,5) 6-8 µm, ocráceas, con poro germinativo hialino, apical y central. Basidios tetraspóricos. Queilocistidios lecitiformes de 23-25 x 10-12 µm con cabeza de 2,5-5 µm de diám. Caulocistidios semejantes a los queilocistidios abundantes en el ápice del pie.

Observaciones: *Conocybe dunensis* se caracteriza por el tamaño de sus esporas, presencia de cistidios lecitiformes en la arista y pie, y por su hábitat psamófilo característico. *C. ammophila* M. Lange fructifica en semejantes áreas y se diferencia por sus esporas de menores dimensiones (Watling, 1982).

Solamente conocemos la cita de Ortega & al. (1991), de la provincia de Almería, aunque la medida de las esporas indicadas por dichos autores es diferente (11-12 x 7-8 µm).

Hygrocybe conica var. ***chloroides*** (Malençon) Bon, Doc. Mycol. 15(59): 52. 1985.

Frecuente junto con *Hygrocybe conicoides* en dunas fijadas, Liencres (Santander), *leg.* G. Moreno, 19-X-1993, AH 16574 y 16575.

Píleo de 3-6 cm de diám., cónico convexo, de color amarillo citrino uniforme o amarillo sin tonos anaranjado rojizos. Láminas blanco amarillentas a amarillentas, libres y con lamélulas. Pie de 4-7 x 0,5-1 cm de color amarillento, blanquecino en la base. Olor y sabor no apreciables. Carne ennegreciendo en la madurez muy lentamente en el sombrero, láminas y pie.

Pileipellis en cutis de hifas cilíndricas, fibuladas, de 2-5 µm de diám. Esporas elipsoidales a faseoliformes,de 10,5-13 (15) x 5-7,5 µm, hialinas. Basidios de 35-45 x 9-11 µm, claviformes y tetraspóricos.

Observaciones: *Hygrocybe conica* var. *chloroides* se caracteriza por el color amarillento de sus cuerpos fructíferos, láminas amarillentas y hábitat psamófilo.

Hygrocybe conicoides P. D. Orton, es una especie que fructifica en los mismos hábitats, incluso a veces juntos. Se diferencia por presentar las láminas de color rojizo a asalmonado y el ennegrecimiento rápido de sus cuerpos fructíferos.

Hygrocybe konradii Haller, se diferencia por fructificar en praderas calizas y poseer esporas más anchas (9-11 µm.).

Hygrocybe persistens (Britzelm.) Singer, se diferencia por fructificar en humus de bosque mediterráneo o en praderas y los basidios son bispóricos.

Hygrocybe persistens var. *cuspidata* (Peck) Arnolds (= *H. aurantiolutescens* P. D. Orton), comparte el hábitat psamófilo y coloraciones amarillentas en sus cuerpos fructíferos, pero estos no ennegrecen (Arnolds, 1990).

En la zona de estudio, al igual que Orton (1969), hemos observado otras colecciones con colores variados, amarillo-anaranjados, láminas amarillas, que en un principio podrían tratarse como *H. persistens* var. *cuspidata*. Sin embargo, algunas colecciones (no todas) ennegrecen lentamente lo que las encuadra en *H. conica s. lato*. Debemos precisar con nuevas recolecciones estos extremos, y observar la variación de coloraciones del basidiocarpo con el grado de hidratación y con la insolación.

Hygrocybe conicoides P. D. Orton, Trans. Br. Mycol. Soc. 43: 262-263. 1960.

Muy frecuente y copioso junto con *Hygrocybe conica* var. *chloroides* en duna fijadas, Liencres (Santander), *leg*. G. Moreno, 30-X-1992, AH 16625; *Ibidem*, 19-X-1993 y 20-X-1993, AH 16627 y 16626.

Observaciones: Esta especie es muy parecida a *Hygrocybe conica* (Schaeff.: Fr.) P. Kumm., se diferencia por presentar un color rojizo a asalmonado en sus láminas tanto en la juventud como en la madurez y un hábitat psamófilo característico.

Inocybe arenicola (R. Heim) Bon, Doc. Mycol. 12 (48): 44 (1982) 1983. (Fig. 3)
≡*I. fastigiata* f. *arenicola* R. Heim, Genre Inocybe:178. 1931.

Frecuente en dunas fijadas cerca de *Pinus pinaster*, Liencres (Santander), *leg*. G. Moreno, 19-X-1993, AH 16511.

Píleo de 3-7 cm de diám., convexo-campanulado ▪ campanulado, con un amplio mamelón central, de color crema pajizo a crema ocráceo, más claro a blanquecino en el mamelón, con fibrillas longitudinales adpresas. Margen incurvado a plano no rimoso. Láminas de color blanquecino, amarillo-crémeo a pardo-ferruginoso. Pie de 3-6 x 0,5-1,5 cm, cilíndrico a veces bulboso, de color blanquecino a crema pálido, enterrado en la arena al menos 2/3 de su longitud.

Pileipellis en cutis formada por hifas fibuladas, cilíndricas, con pigmento intracelular. Esporas elipsoidales a faseoliformes, de 12-15,5 x 6-8 µm, amarillentas y lisas. Basidios de 35-50 x 12-15 µm, tetraspóricos. Queilocistidios de 48-70 x 10-15 µm, cilíndricos, flexuosos. Caulocistidios semejantes a los queilocistidios.

Observaciones: *Inocybe arenicola* nos recuerda a *I. fastigiata*, pero las láminas sin tonos oliváceos en la madurez, el margen pileico no rimoso y hábitat en dunas costeras lo diferencian claramente.

Bon (1983) es el primer autor que considera la variedad de Heim como una especie independiente. Kuyper (1986) en su monografía europea mantiene este tratamiento.

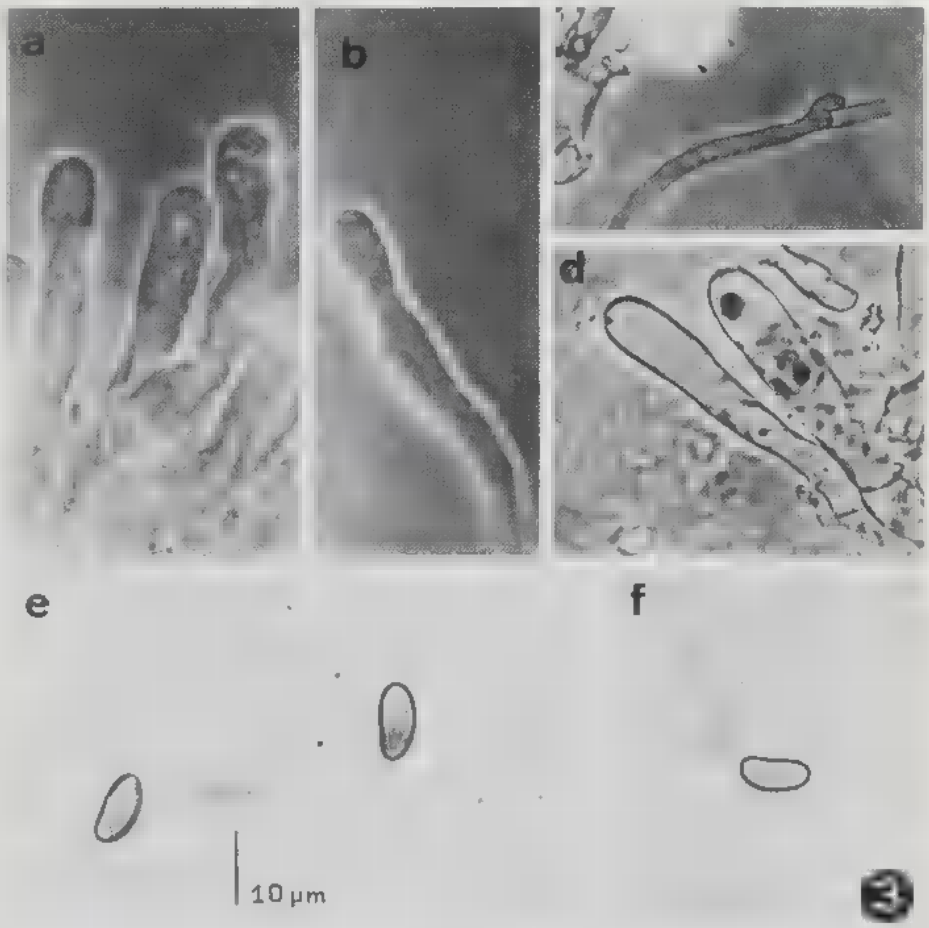


Fig. 3. - *Inocybe arenicola* (AH 16511); a-b y d: queilocistidios; c: fíbulas; e-f: esporas.

Lepiota brunneolilacea Bon & Boiffard, *Bull. Soc. Mycol. France* 88: 18. 1972. (Fig. 4)

Frecuente en dunas marítimas, directamente en la arena cerca de *Ammophila arenaria, Euphorbia paralias*, Liencres (Santander), *leg.* G. Moreno, 19-X-1993,AH ; *Ibidem* 20-X-1993, AH 16510; *Ibidem* Playa de Bolonia, Tarifa (Cádiz). *leg.* I. Pereira, 22-XII-1993, AH 16577; *Ibidem* Ribera de Cabanes (Castellón), *leg.* J. Ayllón & G. Moreno, 6-XII-1993, AH 16511.

Píleo de 3-5,5 cm de diám., convexo a plano convexo, mamelonado, escamoso de color pardo rojizo a pardo lilacino, mamelón más oscuro. Margen plano, excedente. Pie de 4-5,5 x 0,5-1,5 cm, cilíndrico, recto, ensanchandose hacia la base, de color blanquecino con tonos liláceos o vino burdeos sobre todo hacia el ápice, el resto es

Fig. 4. - *Lepiota brunneolilacea* (AH 16510); a-d: queilocistidios; e-f: esporas.

concoloro con el sombrero. Anillo más o menos bien delimitado rojizo oscuro. Carne blanquecina.

Pileipellis formada por una tricodermis de hifas de longitud variable, alargadas con un sustrato basal himeniforme, pigmento intracelular presente. Esporas ovales a elipsoidales de 9-11 (12) x 5-6 µm, hialinas y dextrinoides. Queilocistidios claviformes. Fíbulas presentes.

Observaciones: *Lepiota brunneolilacea* se caracteriza por su porte medio, carnoso, tonalidades liláceas o vinosas en la pileipellis y ápice del pie, anillo sencillo y hábitat en dunas litorales. Es una especie frecuente, según hemos podido comprobar personalmente en las costas españolas peninsulares.

Esta especie se conoce de Francia e Italia y ha sido descrita e iconografiada recientemente en las obras de Candusso & Lanzoni (1990) y Bon (1993).

Una excelente fotografía ha sido publicada por Lanzoni & Candusso (1983).

En España solo conocemos la cita de Cataluña, con una excelente fotografía de J. Carbí, lám. 576 (Bolets de Catalunya, XII colección, 1993).

Marasmiellus mesosporus Singer, Mycologia 65:469. 1973.
=*M. dunensis* Robich, G. Moreno & Pöder, Mycotaxon 42:181. 1991.

Copioso en rizomas y restos herbáceos de *Sporobolus pungens (Poaceae)*. Ribera de Cabanes (Castellón), *leg.* G. Moreno & A. Burguete, 10-XI-1990, AH 12680.

Observaciones: *Marasmiellus mesosporus*, es una especie muy poco citada en la literatura micológica, y siempre sobre diversas *Poaceae* en dunas litorales.

En Europa se conoce de unas pocas localidades de España e Italia, (Robich & al.,1991; 1994)

Marasmiellus trabutii (Maire) Singer, Lilloa 22: 300 (1949) 1951. (Fig. 5)
= M. caespitosus (Pat.) Singer, Pap. Michigan Acad. Sci. 32: 129 (1946) 1948

Muy abundante, disperso o en pequeños fasciculos (2-4 cuerpos fructíferos) sobre tallos de *Juncus maritimus*, Ribera de Cabanes, Castellón, *leg.* G. Moreno & A. Burguete, 9-XI-1990, AH 16199.

Observaciones: *Marasmiellus trabutii*, se caracteriza por el sombrero blanquecino con fibrillas oliváceas a obscuras, las láminas distantes, anastomosadas de color blanquecino, el pie cilíndrico con tonos oliváceos oscuros en toda su longitud y hábitat característico.

Ha sido descrito ampliamente por Honrubia (1984) y Antonín & Noordeloos (1993). Las esporas de nuestra recolección se corresponden con la morfología indicada por Antonín & Noordeloos (*loc. cit.*), para *Marasmiellus trabutii* var. *longisporus* Bas & Noordel., sin embargo las medidas se corresponden con *M. trabutii* var. *trabutii*, lo que confirma la variabilidad de la especie.

En España es un taxon que pasa desapercibido por su pequeño tamaño y ecología particular. Ha sido citado anteriormente por Honrubia (loc. cit.).

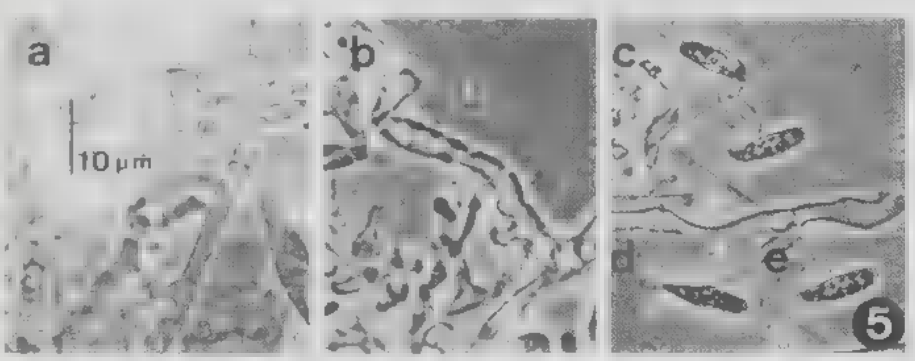


Fig.5. - *Marasmiellus trabutii* (AH 16199); a-b: pileipellis; c-d: esporas.

Marasmius epiphyllus (Pers.: Fr.) Fr., Epicr.:386. 1838. (Fig. 6)
= *M. epiphyllus* var. *plantaginae* R. Heim, Treb. Mus. Cien. nat. Barc. 15 (3): 89. 1964.

Muy abundante sobre hojas secas aún en la planta y vivas de *Plantago crassifolia*, Ribera de Cabanes, Castellón, *leg.* J. Ayllón & G. Moreno, 11-XI-1990, AH 16630; *Ibidem*, Plat de Llobregat (Barcelona), *leg.* A. Mayoral, R. Pöder, J. Boada, C. Iliana & G. Moreno, 29-X-1993, AH 16629.

Observaciones: A nivel macro y microscópico coinciden nuestras recolecciones con *Marasmius epiphyllus*. Es una especie muy abundante en hojas de

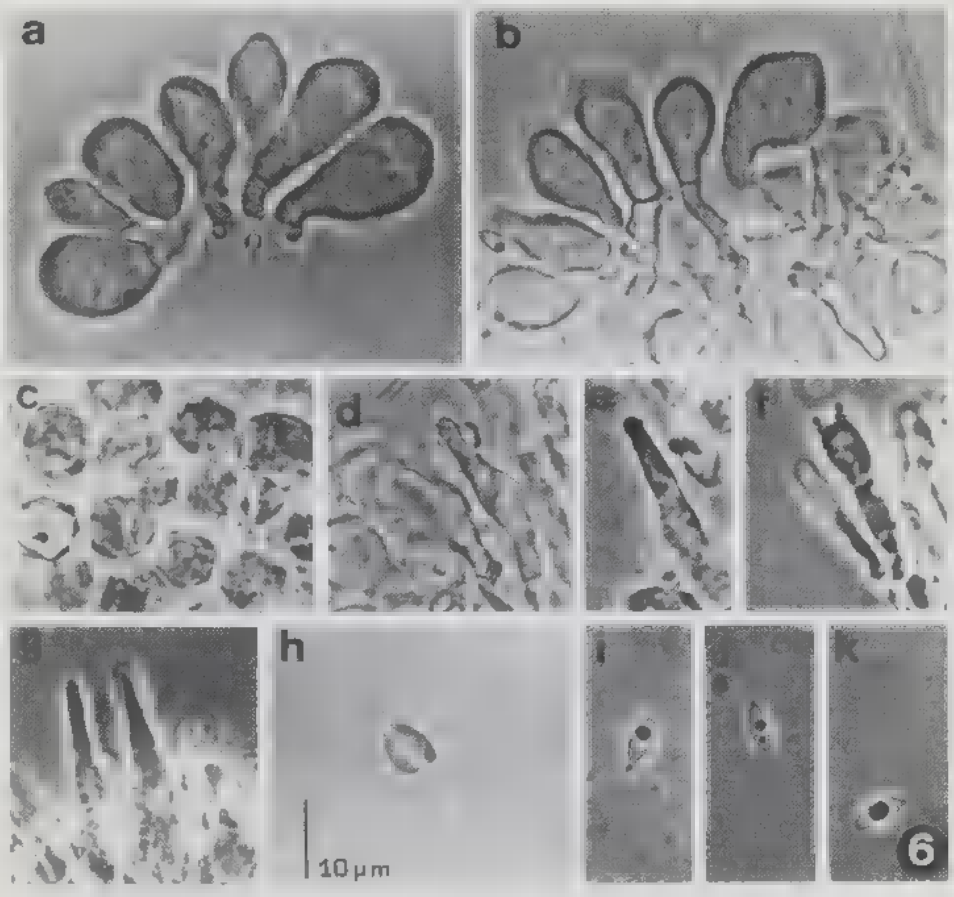


Fig. 6. - *Marasmius epiphyllus* (AH 16630); a-c: pileipellis; d: células de la pileipellis y pileocistidio; e y g: pleurocistidios; f: basidio; h-k: esporas.

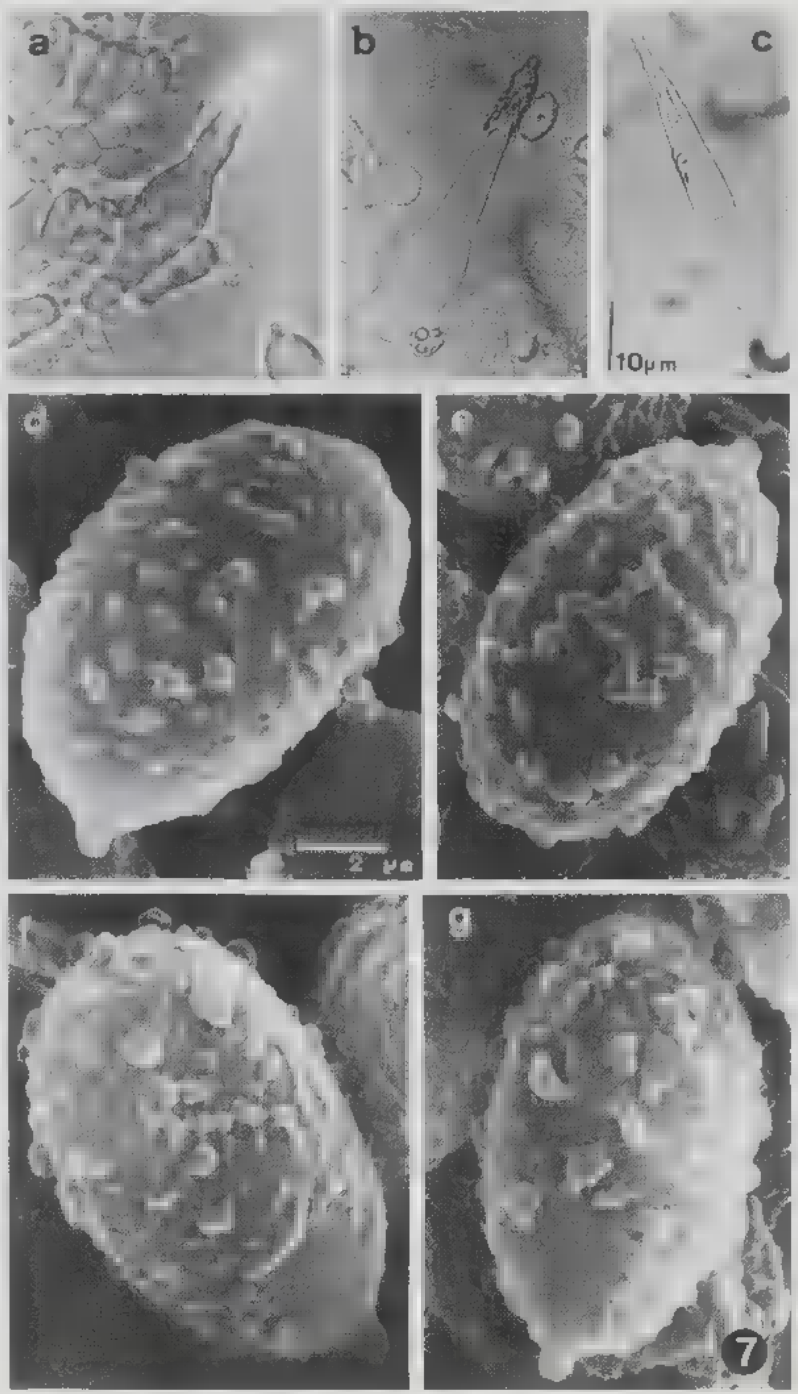
Plantago crassifolia, en áreas marítimas, sobre todo en el otoño cuando la humedad ambiental es alta.

Coincidimos con el tratamiento taxonómico de Antonín & Noordeloos (1993) al sinonimizar la variedad *plantaginae* con la variedad tipo de la especie.

Tal como indican Rivas-Martínez & Losa-Quintana (1969), en España es una especie típica de la asociación *Crucianelletum maritimae*

Melanoleuca polioleuca (Fr.) Kühner & Maire, Bull. Soc. Mycol. France 50: 18.1934. (Fig. 7)

Aislada o dispersa en dunas marítimas con *Ammophila arenaria*, Liencres (Santander), *leg.* G. Moreno, 19-X-1993, AH 16516; *Ibidem*, 20-X-1993, AH 16573; *Ibidem*, Oyambre (Santander), *leg.* G. Moreno, 20-X-1993, AH 16571.



Píleo de 4-6 cm de diám., convexo, plano convexo a ciatiforme de color gris pardo más claro hacia el margen, más oscuro en el centro, no estriado, afieltrado a la lupa. Láminas blanquecinas a color crema pálido, apretadas con lamélulas. Pie de 4-6 x 0,3-0,4 cm, concoloro, cilíndrico con la base engrosada. Carne al corte blanco parduzca por encima de las láminas, parda en el centro del píleo a parda muy obscura o negruzca en el pie y sobre todo en su base.

Epicutis formada por hifas entremezcladas de 10-15 µm de diám., y sin fíbulas. Esporas elipsoidales de 6,5-10 x 4-5,5 µm, verrugosas, amiloides. Al MEB aparecen constituidas por verrugas que se unen en cortas crestas, que a veces se unen en un pequeño retículo. Basidios claviformes tetraspóricos. Pleurocistidios y queilocistidios abundantes, de 50-70 x 8-12 µm., de morfología variable, fusiformes a lageniformes, generalmente no tabicados, con cristales en el ápice. Caulocistidios semejantes a los cistidios himeniales.

Observaciones: Las recolecciones estudiadas se caracterizan por sus cuerpos fructíferos de porte medio a grande, cistidios fusiformes a lageniformes que la encuadran en el subgénero *Macrocystis* Boekhout, sect. *Strictipedes* Bon y esporas alargadas (Q=1,6-1,8). Estos caracteres la encuadran en *Melanoleuca polioleuca* grex.

Aceptamos el sensu estricto para esta especie indicado por Bon (1991), y no amplio y sumamente variable como Boekhout (1988), que incluye en ella *M. vulgaris* Pat., taxon parecido microscópicamente pero muy diferente por el color blanquecino a blanco crémeo de la carne del pie al corte, carácter que se manifiesta constante en las recolecciones españolas. La coincidencia de características microscópicas de *M. vulgaris* con *M. polioleuca*, nos hacen proponerla como una nueva variedad de *M. polioleuca* especie prioritaria. Preferimos crear una variedad nueva para *Melaleuca vulgaris* Pat., Hyménomycètes d' Europe: 96. 1887, especie cistidiada, que fue confundida posteriormente por el propio Patouillard con una especie acistiada, *Melanoleuca vulgaris* (Pat.) Pat., Catal. rais. pl. cellul. Tunésie :22.1897, taxón este último que se corresponde con *Melanoleuca melaleuca* (Pers. : Fr.) Murrill, epíteto prioritario.

La nueva variedad la denominamos: *Melanoleuca polioleuca* var. *confusa* G. Moreno, *nov. var., differt* a *M. polioleuca colore carnis stipitis albido a cremeo.* Humus de *Pinus sylvestris*, Candelario (Salamanca), *leg.* M. Ladero, 25-XI-1989, AH 16706 ***Holotypus***

Melanoleuca albifolia Boekhout, es una especie muy próxima microscópicamente, diferenciándose por sus láminas más blancas y el sombrero muy oscuro (rojizo pardo a sepia oliva pardo).

Melanoleuca cinereifolia (Bon) Bon y su var. *maritima* (Huijsman) ex Bon, son táxones muy próximos en sus caracteres microscópicos con *M. polioleuca*, pero se separan por no presentar la carne del pie obscura

Melanoleuca excissa (Fr.) Singer *s.* Kühner, descrita en Breitenbach & Kränzlin (1991), posee la carne del pie con características similares a nuestra especie, pero presenta el sombrero de color grisáceo uniforme y los cistidios son más estrechos.

Fig. 7. - *Melanoleuca polioleuca* (AH 16571); a-c: pleurocistidios; d-g: variación de la ornamentación y placa suprahilar lisa (técnica del punto crítico).

Omphalina galericolor (Romag.) Bon, Doc. Mycol. 19: 22. 1975. (Fig. 8)
≡ *Omphalia galericolor* Romagn., *Rev. Mycol.* 17: 42.1952.

En grupos dispersos a gregarios, entre briófitos, en dunas marítimas fijadas, Liencres (Santander), *leg.* G. Moreno, 19-X-1993, AH 16513.

Píleo de 0,5-2 cm de diám., infundibuliforme, de color pardo a pardo miel, estriado por transparencia, higrófanos. Margen incurvado. Láminas de color crema, apretadas, decurrentes y con lamélulas. Pie de 1-3 x 0,1-0,3 cm, cilíndrico, central, concoloro o más claro que el píleo.

Fig. 8. - *Omphalina galericolor* (AH 16513); a-c: pileipellis fibulada con pigmento en placas; d: basidiolos; f-g: esporas.

Pileipellis formada por hifas cilíndricas, fibuladas, de .10-15 µm de diám, con pigmento parietal en placas que le dan un aspecto cebrado. Esporas elipsoidales, de 6-10(11) x 4-5 (6) µm, hialinas, no amiloides ni dextrinoides. Basidios tetraspóricos, largamente claviformes.

Observaciones: *Omphalina galericolor* se caracteriza por los colores pardos, sombrero higrófano y estriado, láminas decurrentes y por fructificar en briófitos de dunas litorales fijadas.

Solamente conocemos la cita de Cataluña, con una excelente fotografía de J. Carbó, lám. núm. 585 (Bolets de Catalunya, XII colección, 1993).

Psathyrella ammophila (Durieu & Lével.) P. D. Orton, *Trans. Brit. Mycol. Soc.* 43:180.1960.

En dunas fijadas con *Cynodon dactylon* (Poaceae), Playa de los Genoveses (Almería), *leg.* G. Moreno, E. Gallego, C. Illana & A. Altés, 24-II-1994, AH 16621.

Observaciones: Especie saprófita sobre restos de *Poaceae*, frecuentemente *Ammophila arenaria*, en áreas litorales de la Península Ibérica.

Rhodocybe malençonii Pacioni & Lalli, Doc. Mycol. 14 (56): 56. 1984. (Fig. 9)
= *Rhodocybe ammophila* (Malençon) Pacioni & Lalli, Micol. Ital. 13: 78. 1984, *non R. ammophila* Horak, *Sydowia* 31 :61. 1978.
= *Clitopilus ammmophilus* Malençon, Champignons supérieurs de Maroc 2: 19-20. 1975.

Un ejemplar aislado en dunas marítimas con *Salsola kali, Juncus maritimus, Eryngium maritimum* y *Juniperus oxycedrus* subsp. *macrocarpa*, Playa de Bolonia, Tarifa (Cádiz), *leg.* I. Pereira, 22-XII-93, AH 16512; Un ejemplar aislado junto a Ammophila arenaria, Punta Entinos (Almería), leg. C. Illana, G. Moreno & A. Altés, 15-II-1994, AH 16628.

Sombrero de 3,5 cm de diám., plano convexo, de color blanquecino a gris pardo claro, a gris pardo oscuro, según la insolación o que se encuentre más o menos enterrado en la arena. Margen plano a recurvado. Láminas de color crema, de arista sinuosa, decurrentes y con lamelulas. Pie de 3 x 1,1 cm, ligeramente excéntrico, de color blanquecino grisáceo, radicante, ensanchándose hacia la base que se hace bulbosa. Carne blanquecina. Olor no apreciable. Sabor amarescente.

Pileipellis en cutis formada por hifas alargadas sin fíbulas y con pigmento parietal en placas. Esporas de (6,5) 7,5-9 x 6-7 (8) µm de contorno irregular. Al MEB aparecen anchas verrugas de distribución irregular. Basidios tetraspóricos de 30-37 x 8-9 µm. Cistidios faciales y marginales no observados.

Observaciones: *Rhodocybe malençonii* se caracteriza por el sombrero blanquecino a grisáceo, pie radicante ensanchado inferiormente, espora de contorno irregular y hábitat psamófilo en dunas costeras.

Aparece citado de Cataluña (Tarragona), con una excelente fotografía de M. Tabarés (Bolets de Catalunya, VII colección, 1988) y de Andalucía (Almería) por Ortega & al. (1991).

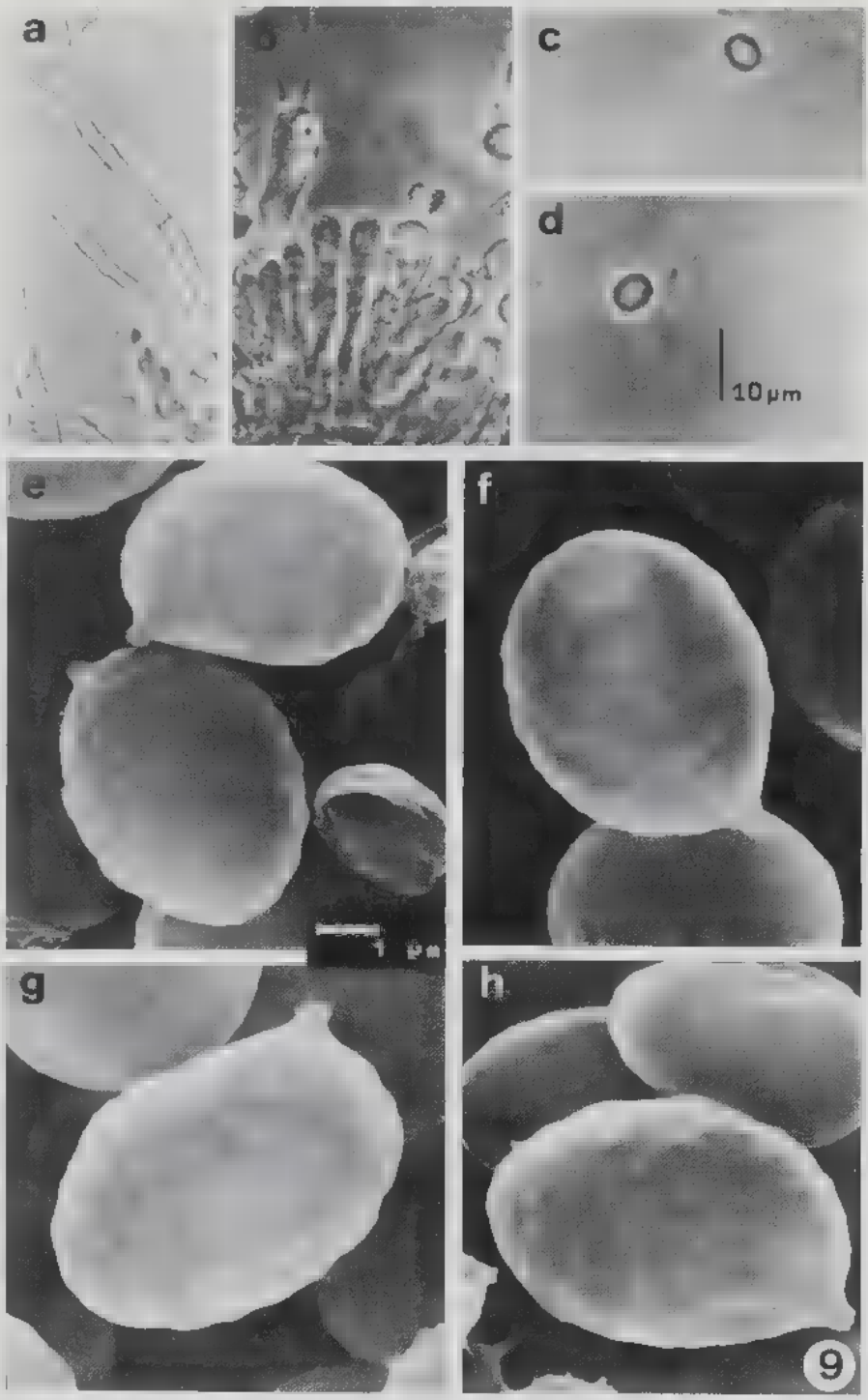


Fig. 9. - *Rhodocybe malençonii* (AH 16512): a: hifa de la pileipellis: b: basidios: c-d: esporas; e-h: variación de la ornamentación esporal (técnica del punto crítico).

AGRADECIMIENTOS

Uno de nosotros (GM) agradece a la Sociedad Micológica Cántabra (Srs. A. Pérez, J. L. Alonso y V. Castañera) la invitación para participar en sus Jornadas Micológicas. Trabajo en parte financiado por el Proyecto de Investigación ref. 93/10, concedido por la Universidad de Alcalá de Henares. Los autores agradecen a los Drs. M. Heykoop y F. Esteve-Raventós sus comentarios y aportación bibliográfica.

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DESCOLEA MACULATA BOUGHER (AGARICALES), NUEVA CITA PARA EUROPA

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RESUMEN - Se describe e ilustra *Descolea maculata* Bougher, especie australiana que representa la primera cita del género y de la especie en Europa. Fructifica en *Eucalyptus globulus* en repoblaciones alóctonas en Galicia, en el noroeste de España.

RÉSUMÉ - Description et illustration de *Descolea maculata* Bougher, espèce australienne. Il s'agit de la première citation de ce genre et de cette espèce en Europe. *Descolea maculata* fructifie sur *Eucalyptus globulus*, introduits en Galice et dans le Nord Est de l'Espagne.

ABSTRACT - Description and illustration of *Descolea maculata* Bougher, Australian species. First mention of the genus and the species in Europe. *Descolea maculata* was fruiting on *Eucalyptus globulus* introduced in Galicia and in North West Spain.

KEY-WORDS - *Descolea maculata*, chorology, ecology, taxonomy.

INTRODUCCIÓN

El género *Descolea* descrito por Singer (1951), para *D. antarctica* Singer, se caracteriza principalmente por sus cuerpos fructíferos medios a pequeños, con un anillo estriado, por su pileipellis himeniforme, por sus esporas ocráceas, citriformes, ornamentadas y por fructificar en *Nothofagus spp.*

Posteriormente se ha recogido sobre diversos sustratos, principalmente en los bosques subantarticos del hemisfério Sur, en la India (Himalaya) y en la región más oriental de Eurasia (Siberia y Japón) en *Pinaceae* (*Abies, Pinus* y *Taxus*), *Fagaceae* (*Quercus, Castanopsis* y *Nothofagus*) y *Myrtaceae* (*Eucalyptus, Leptospermum* y *Melaleuca*) principalmente. En Nueva Zelanda fructifica en bosques de *Nothofagus* (*Fagaceae*) y *Leptospermum* (*Myrtaceae*); en Japon en *Pinus* y *Larix* (*Pinaceae*) y en *Quercus* y *Castanopsis* (*Fagaceae*) (Horak, 1971). En la India en bosques de coníferas puras de montaña (*Abies, Pinus, Picea* y *Taxus*).

Su comportamiento como un hongo ectomicorrizógeno facultativo o saprófito habia suscitado controversias desde su creación, Bougher & Malajczuk (1985) en recolecciones sobre *Eucalyptus* spp. confirman su comportamiento ectomicorrizógeno.

Descolea es un género que se puede confundir con especies de *Pholiotina* Fayod y con *Rozites* P. Karst., y que según Hawksworth & al. (1983), es un representante de la familia *Cortinariaceae*. Sin embargo, Singer (1986), lo considera incluido en la familia *Bolbitiaceae*. Amplia información al respecto podemos encontrarla en el trabajo de Horak (1971).

Actualmente se conocen descritas nueve especies de este género, que son: *Descolea antarctica* Singer y *D. pallida* Horak (en *Nothofagus* en el sur de América), *D. majestica* Horak (en *Nothofagus* en Nueva Zelanda), *D. recedens* (Cooke & Massee) Singer (en *Eucalyptus* en Australia), *D. gunnii* (Berk.) Horak (en *Nothofagus* y *Leptospermum* en Nueva Zelanda), *D. pretiosa* Horak (en *Abies, Pinus* y *Taxus* en el Himalaya, India), *D. flavo-annulata* (Vasilieva) Horak (en *Larix, Pinus, Quercus* y *Castanopsis* en Siberia , Japón y Corea), *D. rheophylla* (Bertault & Malençon) Malençon (en repoblaciones de *Eucalyptus* en Marruecos) y *D. maculata* Bougher (en *Eucalyptus* de Australia), y una especie innominada descubierta recientemente en las islas Hawaii (Horak & Desjardin, *Agaricales* of the Hawaiian Islands. 3. Mycologia, en prep.).

El género *Descolea*, es un género típico del hemisferio Sur, y su presencia en el hemisferio Norte (España y Marruecos) debe interpretarse como esporádica, y posiblemente se traten de especies alóctonas, importadas con las repoblaciones de *Eucalyptus*, con las semillas y árboles vivos. Así parece confirmarlo la presencia de *Descolea maculata* en España, descrita recientemente de Australia en *Eucalyptus diversicolor* y *E. marginata* (Bougher & Malajczuk, 1985). Sin embargo *D. rheophylla* (Bertault & Malençon) Malençon, aparece en la actualidad como una especie solo conocida en *Eucalytus.globulus* en Marruecos (Malençon, 1979), pero creemos que es muy probable su presencia en Australia.

DESCOLEA MACULATA BOUGHER, Aust. J. Bot. 33: 620. 1985.

(Figs. 1-3)

Material estudiado: ESPAÑA: Galicia, Pontevedra, zona costera ria de Arosa, en restos de *Eucalyptus globulus*, 6.II.1993, *leg.* M. Lago, (AH 16707, ZT 5323). AUSTRALIA: Western Australia: Perth, Floreat Park, in glasshouse pots with *Eucaliyptus diversicolor* and *E. marginata*, 17.V.1983, *leg.* N. Malajczuk, (*Isotypus Descolea maculata*, ZT 1843); same region, on soil, under *Melaleuca* sp., 28.VI.1985, *leg.* True (ZT 2762).

Sombrero de (1)1,5-3,5 (5) cm de diám., convexo a plano convexo, con un amplio mamelón obtuso central, de color pardo dátil a pardo rojizo, con tintes o irisaciones doradas, con escuamulas muy pequeñas a subliso, sin fibrillas, seco, estriado a debilmente acanalado, más marcado en periodos húmedos. Láminas adnatas a sublibres, con lamélulas de color ferruginoso a pardo rojizo, más intenso que el sombrero. Pie de 2-4 x 0,2-0,4 cm, cilíndrico a muy ligeramente ensanchado en la base,

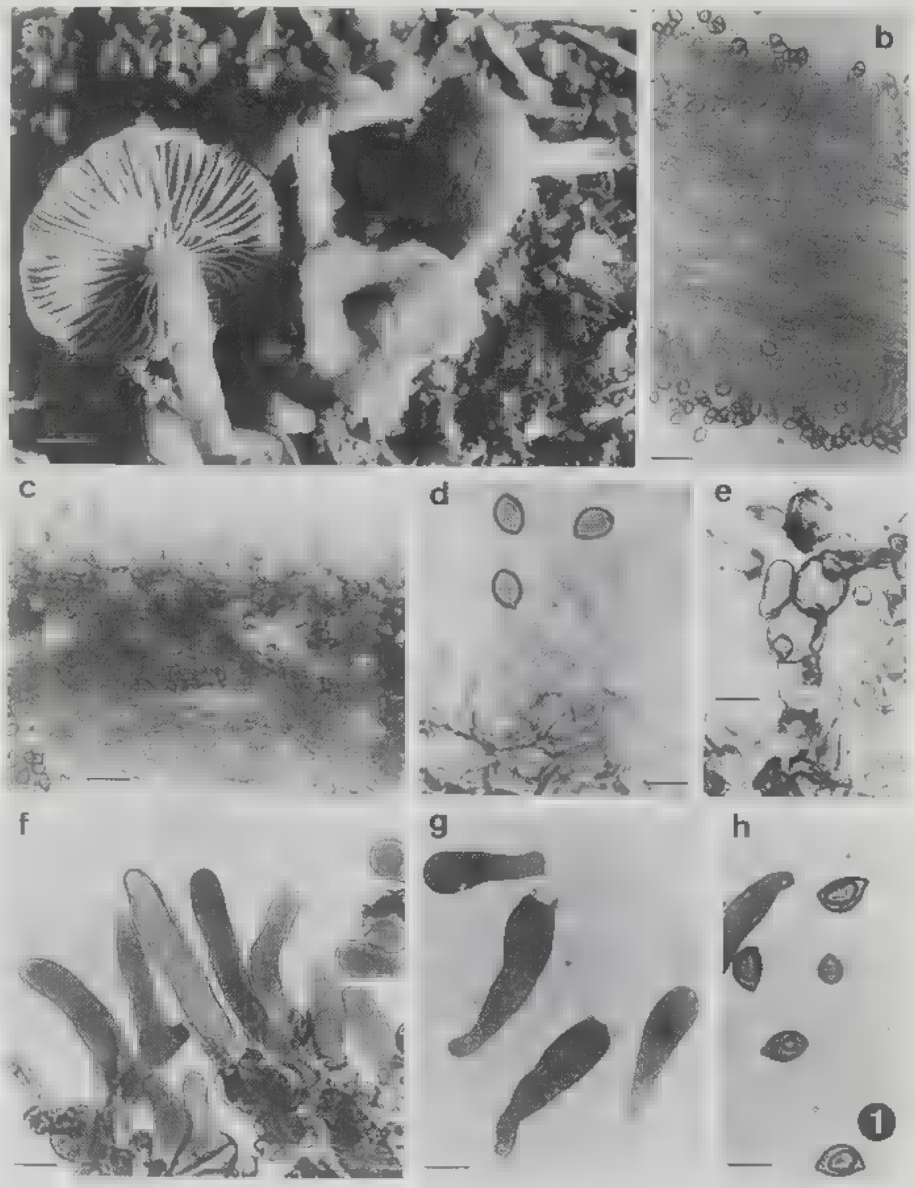

Fig. 1.- *Descolea maculata* AH 16707: a. cuerpos fructíferos. Barra 1 cm; b. trama laminal regular. Barra 20 µm; c. sección de la pileipellis. Barra 20 µm; d.-e. detalle pileipellis. Barra 10 µm; f. queilocistidios. Barra 10 µm; g. basidios. Barra 10 µm; e. esporas. Barra 10 µm.

concoloro al sombrero, fibriloso en el ápice. Anillo membranoso, con tonos amarillo dorados, estriado exteriormente, en la madurez se desprende facilmente del pie, pero no llega a caer.

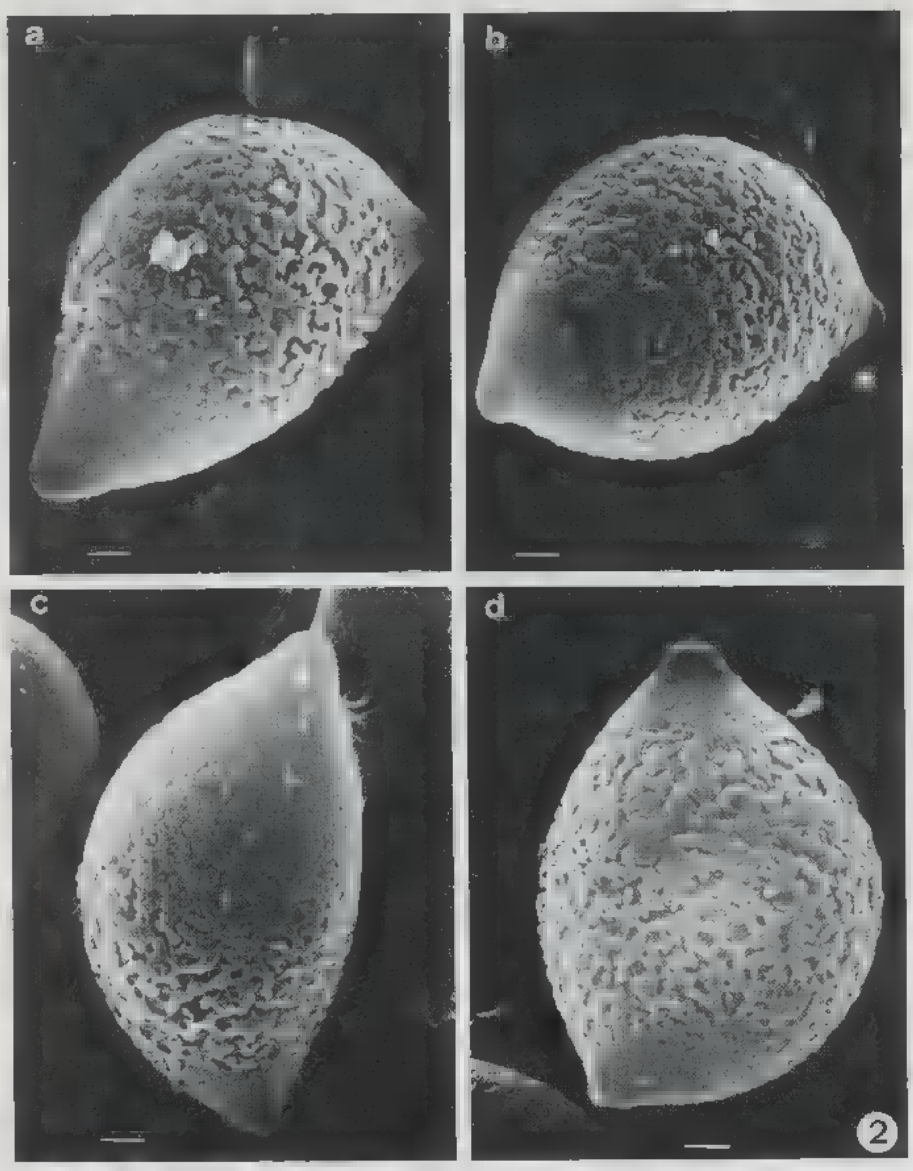

Fig. 2.- *Descolea maculata* AH 16707: a-d. detalle ornamentación esporal al MEB. Barra 1µm.

Esporas de 10,5-12,5 x 6-7 µm (basidios tetraspóricos), -13,5 x -8 µm (basidios bispóricos), amigdaliformes ■ citriformes con una papila apical más o menos marcada, ocráceas, sublisas a ligeramente punteadas al microscopio óptico. Al MEB la

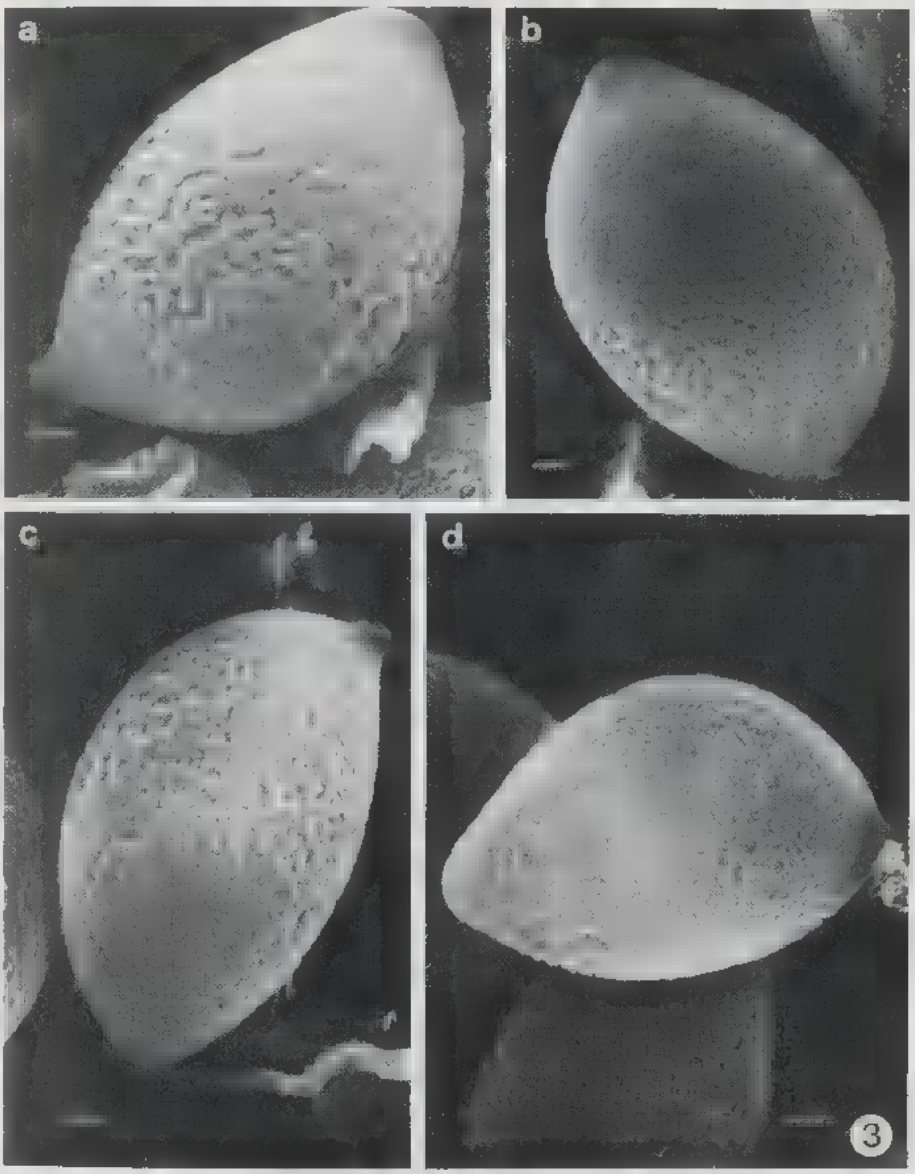

Fig. 3.- *Descolea maculata* ZT 2762: a-b. ornamentación esporal al MEB. Barra 1µm; ZT 1843 *Isotypus*. c-d. ornamentación esporal al MEB. Barra 1µm.

ornamentación esporal aparece formada por cortas crestas y pequeñas verrugas que forman un subreticulo muy poco marcado, carece de placa suprahilar. Basidios de 28-42 x 8-10 µm, claviformes generalmente tetraspóricos raramente bispóricos, a veces con un pigmento amarillo ocráceo plasmatico. Cheilocistidios de 20-55 x 5-10 µm, de

morfología variable, la mayoría cilíndricos o subclavados. Pleurocistidios ausentes. Pileipellis himeniforme a epitelial, formada por células claviformes o globosas en cortas cadenas, de 12-30 µm de diám., con pigmento incrustado o vacuolar amarillo ocráceo. Escamas del velo compuestas de hifas cilíndricas y cortas, de paredes gruesas, fuertemente incrustadas con pigmento pardo amarillo, de 5-10 µm de diám. Trama laminal formada por hifas de 5-10 µm de diám., típicamente regular. Fíbulas presentes.

Observaciones: *Descolea maculata*, fructifica en restos leñosos, hojarasca y tocones de *Eucalyptus globulus*, arbol alóctono introducido en Galicia en diferentes repoblaciones forestales. Esta especie ha sido observada muy abundante (Castro, com. pers.) en toda la provincia de Pontevedra (A. Guarda, Camposancos; Vigo, A. Guia; Redondela, Rande), durante el otoño y el invierno, especialmente en el año 1993.

En el terreno puede confundirse con representantes de *Pholiotina* (*Conocybe*) grupo *blattaria*, pero se diferencia principalmente por la morfología y ornamentación esporal.

Descolea maculata se caracteriza principalmente por su pileipellis formada por células globosas a piriformes, que se reunen en cortas cadenas, y por sus esporas citriformes, sin apenás ornamentación visible al microscopio óptico. Esporas rugulosas hasta lisas han sido descritas en *Descolea phlebophora* Horak, recogida en Nueva Zelanda y Tasmania. En el resto de especies conocidas, la ornamentación esporal observada al microscopio óptico es más fuerte, y está formada por verrugas bien marcadas conectadas por crestas (Bougher, 1987).

El material australiano de *Descolea maculata*, es más robusto que el español (sombreros de 2-5 cm de diám. y pie de 3-6 x 0,3-1,5 cm) y los queilocistidios son ligeramente más cortos y claviformes (15-30 x 5-12 µm), pero el resto de características, sobre todo el hábitat, tamaño esporal y ornamentación son similares. La morfología esporal es variable en el material español donde abundan las esporas citriformes, en el material australiano son más frecuentes las esporas elipsoidales ■ amigdaliformes, sin embargo en todas las muestras estudiadas existen las tres posibilidades (elipsoidales, citriformes y amigdaliformes), en mayor o menor grado. Las pequeñas variaciones morfológicas en las muestras australianas y españolas, no nos parecen suficientemente significativas, y preferimos considerarlas como la misma especie, siendo el caracter más importante y constante en las muestras estudiadas la ornamentación esporal.

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Nuestra agradecimiento a la DGICYT por la concesión del proyecto de investigación nº PB91-0165, dentro del cual se enmarca dicho trabajo. A la Dra. M. Castro por los datos corológicos y fotografía del material estudiado. Al Dr. N. Bougher por el envio de material *Isotypus* de *Descolea maculata*. A J. A Pérez y A. Priego, del Servicio de Microscopía Electrónica de la Universidad de Alcalá de Henares, su apreciable ayuda al microscopio electrónico de barrido. Por último, ■ A. Dreze (Jardin Botanique National de Belgique) por su ayuda en los cortes histológicos.

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SOME SPECIES OF *PYTHIUM* ISOLATED FROM CULTIVATED SOILS IN NORTHERN FRANCE

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ABSTRACT - Nine species of *Pythium* including *P. echinulatum, P. irregulare, P. mamillatum, P. minor, P. ostracodes, P. torulosum, P. oligandrum, P. ultimum*, and *P. rostratum* isolated from cultivated soils in northern France are described. Some of these are new to this country. Taxonomic and morphological details of the 9 species of fungi are discussed.

RÉSUMÉ - Neuf espèces de *Pythium* notamment *P. echinulatum, P. irregulare, P. mamillatum, P. minor, P. ostracodes, P. torulosum, P. oligandrum, P. ultimum*, et *P. rostratum* ont été isolées à partir de sols cultivés dans le nord de la France. Quelques unes d'entre elles s'avèrent être nouvelles pour ce pays. Les détails morphologiques et taxonomiques de ces neuf espèces font l'objet de ce présent article.

KEY WORD - *Pythium*, sporangia, oogonia, antheridia, oospore.

INTRODUCTION

A perusal of the literature on the genus *Pythium* shows that very little work has been done on this important genus in France. Although works on the ecological and pathological aspect of *Pythium* have been carried out (Roze & Cornu, 1869; Moreau & Moreau, 1958; Bouhot, 1975; Montfort & Rouxel, 1988), have a description of *Pythium violae* as the causal organism for the «cavity spot disease» of carrots, and that of Forbes & Davet (1990) on the pathogenicity of *Pythium ultimum, P. sylvaticum*, and *P. irregulare* on soyabean roots, but none of these have a taxonomic treatment of the said species. An attempt is underway towards the taxonomy of the genus *Pythium* in France. A project on the isolation, identification, and preservation of pythiaceous fungi has been undertaken at the university of Bourgogne in Dijon. A number of soil samples from the region of Lille and Compiègne were examined and in this report nine species of *Pythium* are treated. All the cultures of the described fungi are being maintained at the Laboratoire de Mycologie, Université de Bourgogne, Dijon, France.

MATERIALS AND METHODS

Soil samples were collected in sterilized capped bottles and brought to the laboratory. Fungi were isolated by baiting with boiled hemp-seed halves introduced to a

soil suspension in water (Paul, 1986, 1987). Temperature/growth relations were observed on potato carrot agar (PCA) and corn meal agar (CMA). Benomyl (5 mg/l) was used to suppress the growth of *Fusarium* like fungi (Paul, 1991). Identification was done with the help of keys and descriptions of Middleton (1943), Waterhouse (1967), Plaats-Niterink (1981) and Dick (1990).

RESULTS AND OBSERVATIONS

***Pythium torulosum* Coker & Patterson** (Figs. 1-5, & Fig. 59).

Colonies on CMA and PCA submerged, on PCA showing a rosette pattern and growing with an average daily growth rate of 15 mm at 25°C. Main hyphae upto 5-6 µm wide. **Sporangia** consisting of filamentous inflated, toruloid elements, vesicles and zoospores readily formed between 15-20°C, encysted zoospores measure about 7 µm in diam. **Oogonia** terminal or at times intercalary, globose or sub-globose, 12-25 ($\bar{x}$ x 19 ± 0.6) µm diam. **Antheridia** mostly monoclinous, 1-2 per oogonium, antheridial cells making apical contact with the oogonia. **Oospores** globose, plerotic, single, 10-24 ($\bar{x}$,16 ± 0.6) µm diam. Wall 1-2,5 µm in thickness.

Pythium torulosum was isolated only twice from the region of Lille. The above description is that of F-80. Apart from slightly bigger oogonia and oospores, all the other characters of this isolates resembles with the description of *P. torulosum* found elsewhere (Plaats-Niterink, 1981). This is the first taxonomic treatment of the species in France.

***Pythium rostratum* Butler** (Figs 6-9)

Colonies on CMA and PCA submerged, showing a chrysanthemum patten on PCA. It is a slow growing fungus with an average daily rate of 8.5 mm at 25°C on this medium. Main hyphae upto 7-8 µm wide. **Sporangia** globose, ovoid, limoniform to cylindrical, terminal, intercalary, or catenulate, 18-32 µm diam. ($\bar{x}$ 26.3 ± 0.6). **Oogonia** smooth walled, mostly intercalary or in chains, globose, ovoid, limoniform, ellipsoidal, 17-32 ($\bar{x}$ 23.9 ± 0.6) µm diam. **Antheridia** mostly hypogynous, monoclinous and sessile, 1-2 per oogonium. **Oospores** globose, rarely oval, plerotic and aplerotic, usually single, rarely two per oogonium, 16-25 ($\bar{x}$ 19.6 ± 0.6) µm diam. Wall 1.5-3 µm thick.

Pythium rostratum was originally isolated from garden soil in France (Butler, 1907). It is a very common species inhabiting the soil and was isolated from soil samples taken in the Compiègne as well as the Lille areas. Most of the morphological characters of the above described isolate (F-83) resemble to those found in the literature. The only difference worth mentioning is the presence of longer somewhat rectangular sporangia which can attain a length of up to 40 µm instead of 27 µm recorded by Plaats-Niterink (1981).

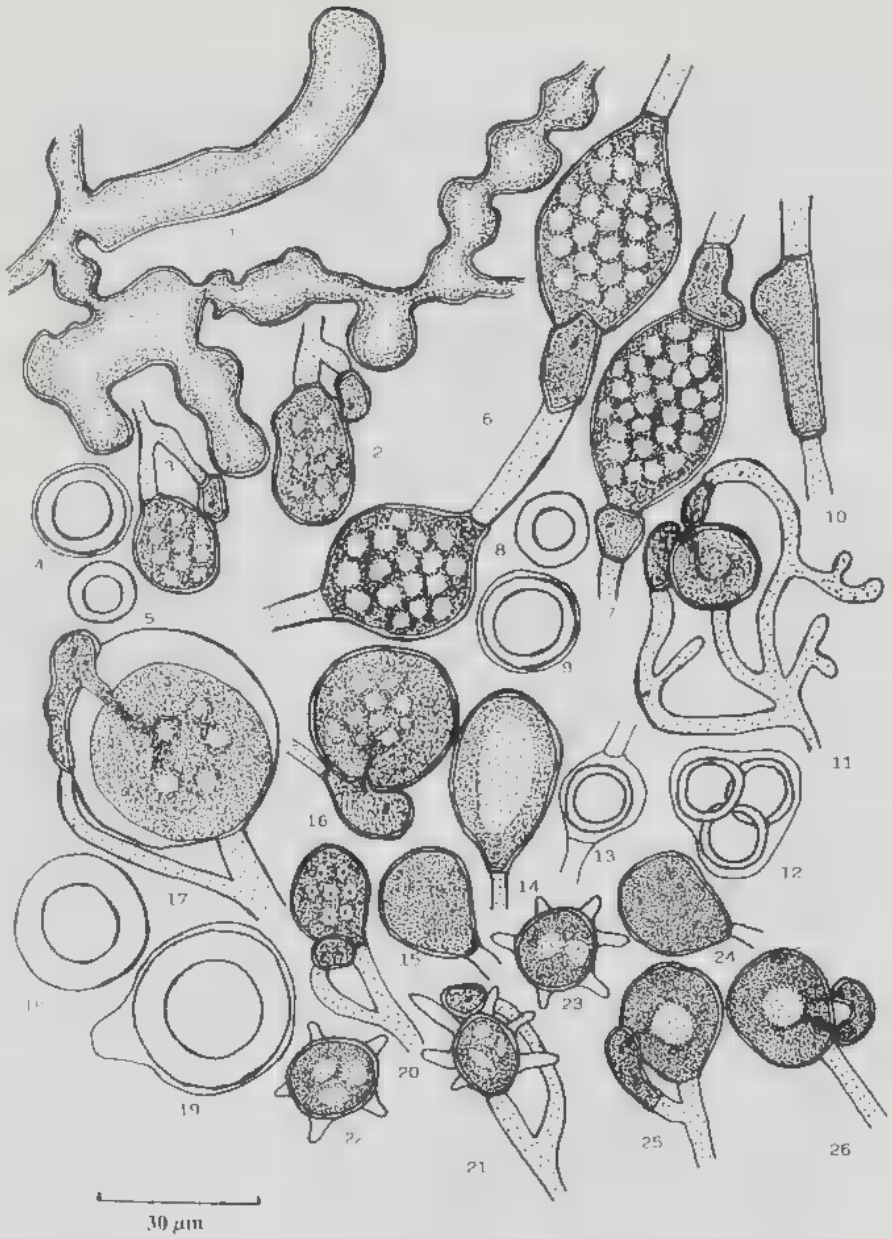
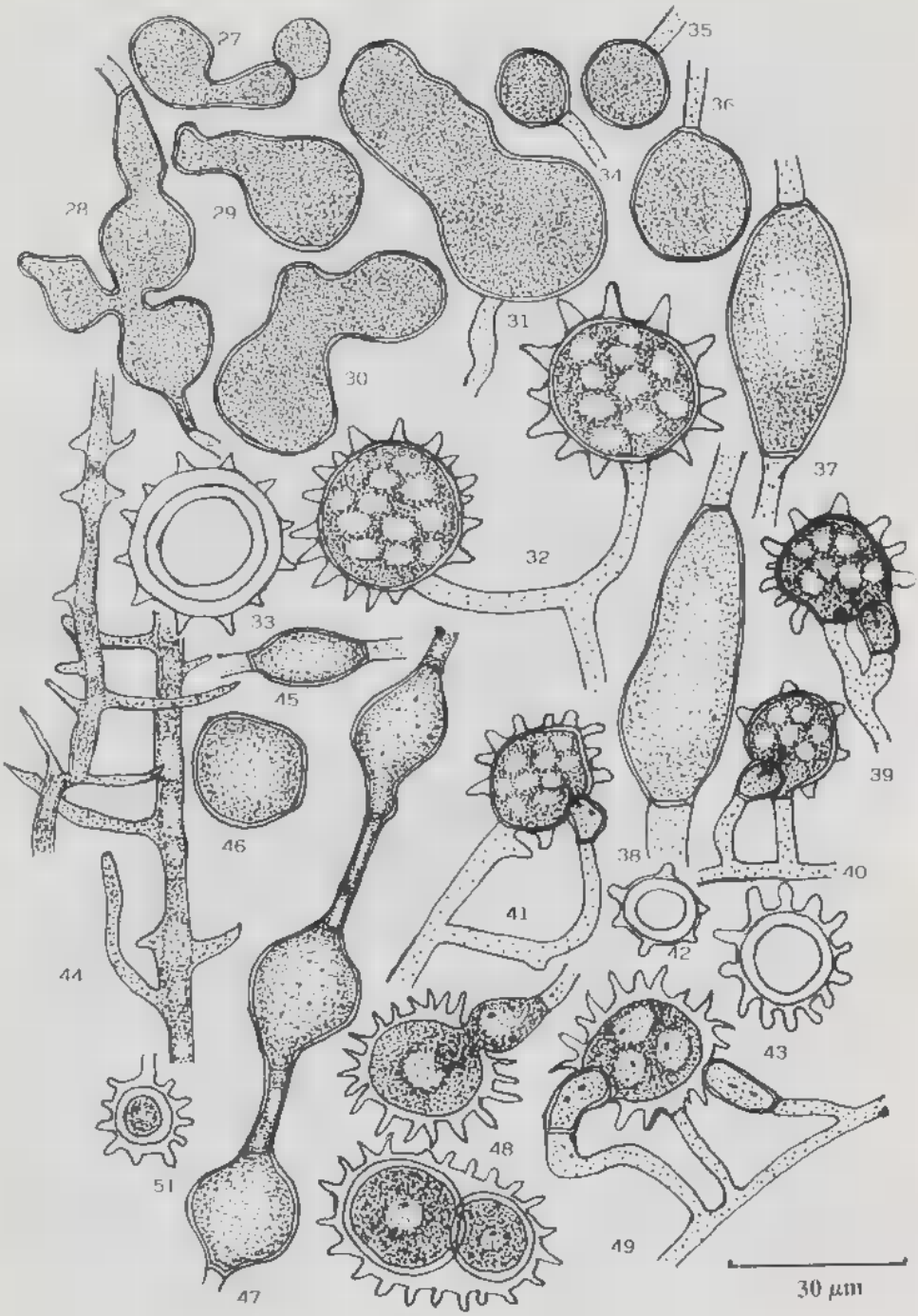


Figure 1-5: *Pythium torulosum*. 1: sporangia, 2-3: oogonia with monoclinous antheridia, 4-5: oospores; Figs 6-9: *Pythium rostratum*. 6: intercalary oogonia, 7: oogonium with hypogynous antheridia, 8-9: oospores; Figs 10-13: *Pythium minor*. 10: hyphal body, 11: oogonia with branched antheridia, 12-13: oospores; Figs 14-19: *Pythium ostracodes*. 14-15: sporangia, 16-17: oogonia:, 14-15: sporangia, 16-17: oogonia with antheridia, 18-19: oospores; Figs 20-23: *Pythium irregulare*. 20: smooth walled oogonia, 21: spiny oogonia with antheridia, 22-23: spiny oogonia; Figs 24-26: *Pythium ultimum*. 24: hyphal body, 25-26: oogonia with antheridia.



***Pythium minor* Ali-Shatayeh & Dick** (Figs 10-13, & Figs 52-53).

Colonies on CMA and PCA submerged, showing an indistinct rosette pattern on PCA. Daily growth rate on PCA at 25°C is 11 mm. Main hyphae upto 6 µm wide. **Sporangia** and zoospores not formed. Hyphal bodies produced abundantly on solid as well as in water on hemp seed halves. These are usually intercalary, catenulate, rarely terminal, globose, ovoid to cylindrical, the elongated ones are at times hardly thicker than the vegetative hyphae but filled with denser protoplasm, measuring 9-20 ($\bar{x}$ 12.4 ± 0.6) µm diam. **Oogonia** terminal, infrequently intercalary, globose, ovoid, smooth walled, measuring 14-25 ($\bar{x}$ 15.9 ± 0.6) µm diam. **Antheridia** monoclinous, much branched,giving a coralloid appearance and growing towards the oogonium, providing 1-2 antheridial cells to the latter. **Oospores** globose, 1-3 per oogonium, plerotic, rarely aplerotic, 10-15 ($\bar{x}$ 12.7 ± 0.6) µm diam. with a very thin wall of 0.5-1 µm. Description isolate no. F-10.

Pythium minor is a very common species in the north of France. It was isolated twice in Compiègne and 5 times from soil samples taken at different places in the region of Lille. After its discovery in England (Ali-Shtayeh & Dick, 1985) it has not been reported from elsewhere. Branched antheridia forming a corolloid structure around the oogonia, small oogonia and oospores, and slow growth makes it easily distinguishable from other species. However there are some differences between this isolate and the one described by Ali-Shtayeh & Dick (1985): the hyphal bodies in this case are much smaller (9-20 µm instead of 20-40 µm). More than 3 oospores per oogonium were not observed in the isolates from France as compared with 6 from that described from England. This is the first report of its occurrence in France.

***Pythium ostracodes* Drechsler** (Figs 14-19, & 54-55).

Colonies on CMA and PCA producing abundant aerial mycelium. Daily growth rate on PCA at 25°C 8-9 mm. Main hyphae upto 7 µm wide. **Sporangia** spherical, ovoid, at times with an apical papilla 13-33 µm diam. ($\bar{x}$ 23 ± 0.6). **Oogonia** smooth walled, globose, terminal or intercalary, 15-40 ($\bar{x}$ 26 ± 0.6) µm diam. **Antheridia** 1-2 per oogonium, monoclinous with long antheridial cells that apply to the oogonium to most of its surface, antheridial cells upto 25 µm long and 6 µm wide. **Oospores** globose, plerotic, one per oogonium, 14-39 ($\bar{x}$ 24 ± 0.6) µm diam., and provided with a very thick wall of 4-7 µm.

Pythium ostracodes isolated only once, from a soil sample taken from a wheat field on Lille (No F-66). It was first described from wheat in Texas (Drechsler, 1943) and later on it was isolated from rhizomes of latus in Japan (Takahashi *et al.*, 1965). This is the first report of its presence in France. This species can be separated from

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Fig. 27-33: *Pythium oligandrum*. 27-31: irregular elements of the contiguous sporangia, 32: terminal oogonia, 33: aplerotic oospores; Figs 34-43: *Pythium mamillatum*. 34-38: sporangia, 39-41: ornamented oogonia with antheridia, 42-43: oospores. Figs 44-51: *Pythium echinulatum*. 44: vegetative hyphae, 45-47: sporangia, 48: oogonia with hypogynous antheridia, 49: oogonia with monoclinous antheridia, 50: oogonia containing two oospores, 51: oogonia with single oospore.

other species by its slow growth, large plerotic oospores, and long, laterally applied antheridia. This isolate has all these characters, but it does not sporulate. Zoospores were not observed, moreover its average oogonial and oosporal sizes are smaller (26 & 24 µm instead of 35 & 32.5 µm, respectively), and oospore wall thicker (upto 7 µm instead of 5 µm) than those described by Plaats-Niterink (1981).

***Pythium irregulare* Buisman** (Figs 20-23).

Colonies on CMA & PCA producing profuse aerial mycelium. Daily growth rate on PCA at 25°C is 24.5 mm. Main hyphae up to 5-6 µm wide. **Hyphal swellings** globose to ovoid, sometimes provided with single papilla, terminal or intercalary, 14-26 µm diam. ($\bar{x}$ 19 ± 0.6). Sporangia and zoospores were not formed. **Oogonia** terminal or intercalary, globose to somewhat elongated, 13-21 ($\bar{x}$ 20 ± 0.6) µm diam., smooth walled or provided with a varying number of blunt conical projections up to 12 µm long, 0-6 per oogonium. **Antheridia** mostly monoclinous, 1-3 per oogonium. **Oospores** globose, plerotic or aplerotic, 12-20 ($\bar{x}$ 16 ± 0.6) diam. Wall 1.5-2 µm thick.

Pythium irregulare was isolated only on three locations, once in Compiègne and twice in the region of Lille. Because of the presence of both spiny and smooth- walled oogonia, it is an easily recognisable fungus. However, in water cultures, the number of ornamented oogonia was larger than that of the smooth walled ones. The features of the three isolates agree well with the description of this species reported in literature (Plaats-Niterink, 1981). The characteristics given above are those of culture no. F-75 isolated from ■ soil sample taken in the region of Lille.

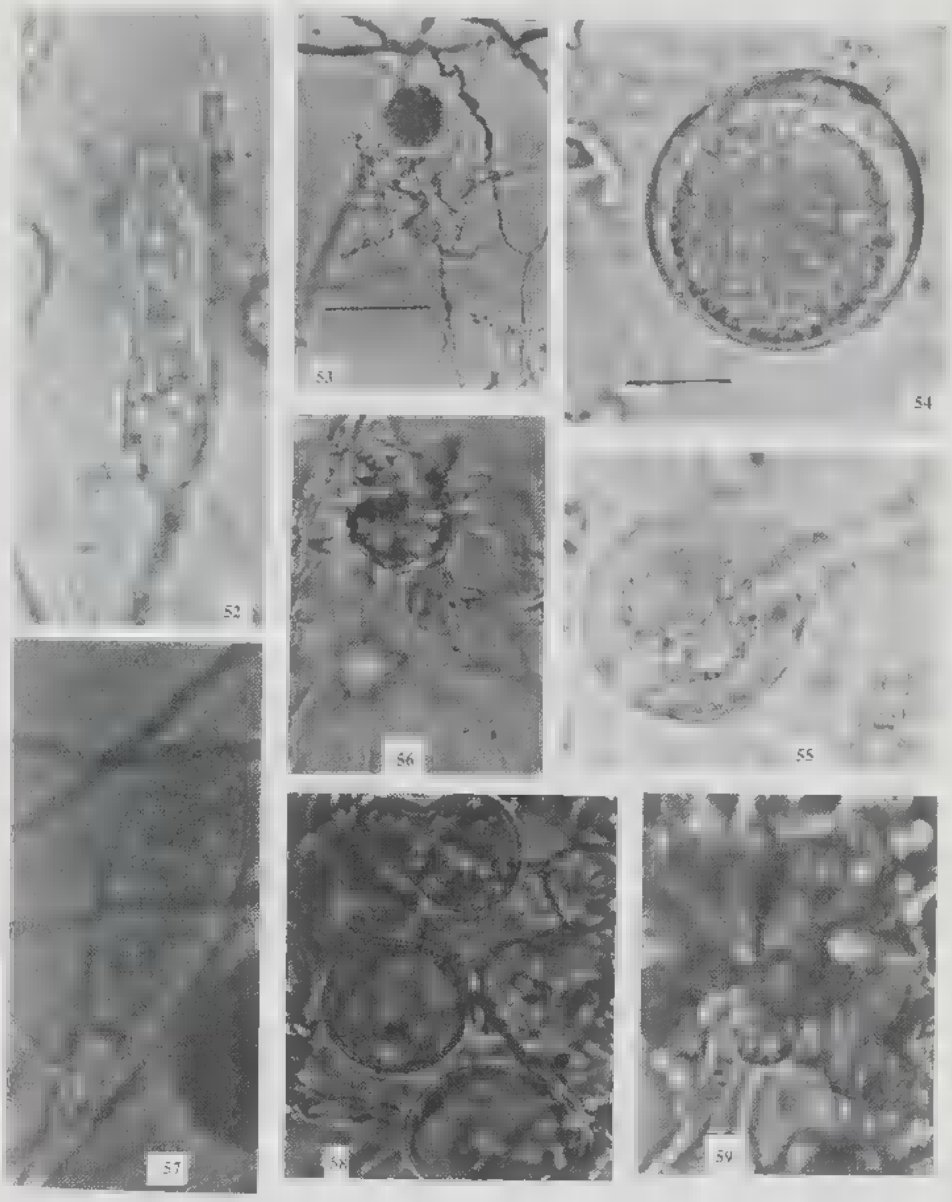
***Pythium ultimum* Trow** (Figs 24-26).

Colonies on CMA forming profuse aerial mycelium and on PCA showing an indistinct radiate pattern. Average daily growth rate on PCA at 25°C is 28 mm. Main hyphae up to 10 µm wide. **Sporangia** and zoospores not formed. Hyphal swellings formed plentifully and are globose, intercalary, sometimes terminal 18-25 µm diam. ($\bar{x}$ 20 ± 0.6). **Oogonia** terminal or intercalary, spherical, smooth walled, 19-31 ($\bar{x}$ 23.4 ± 0.6) µm diam. **Antheridia** mostly monoclinous originating immediately below the oogonium, sessile, saclike, infrequently hypogynous, rarely diclinous, 1-2 per oogonium. **Oospores** spherical, aplerotic, at times plerotic, single, 17-24 ($\bar{x}$ 20.6 ± 0.6) µm diam. Wall 1-3 µm thick.

Pythium ultimum is an aggressive plant parasite and was isolated many times in Compiègne as well as Lille. The above description is that of isolate F-39.1 isolated from Lille. Inspite of some variations of oogonium and oospore dimensions, and the presence of some plerotic oospores together with the usual aplerotic ones, all the other characters of this fungus fit closely the description of *P. ultimum* found in the literature (Plaats-Niterink, 1981).

***Pythium oligandrum* Drechsler** (Figs 27-33 & Fig. 58)

Colonies on CMA forming some aerial mycelium, submerged on PCA showing an indistinct radiate pattern. Average daily growth rate on PCA at 25°C is 25 mm. Main hyphae upto 8 µm wide. **Sporangia** intercalary, composed of spherical to irregular, continguous elements of up to 35 µm wide and 60 µm long. Zoospores not formed. **Oogonia** mostly terminal, spherical, ornamented with conical, acutely tipped spines,



Figs 52-53: *Pythium minor*. 52: elongated hyphal body, 53: oogonia with branched antheridia; Figs 54-55: *Pythium ostracodes*. 54: oogonia with antheridia, 55: plerotic oospore; Figs 56-57: *Pythium echinulatum*. 56: oogonia with hypogynous antheridia, 57: elongated intercalary sporangia; Fig. 58: *Pythium oligandrum*. contiguous sporangia, Fig. 59: *Pythium torulosum*: inflated elements of sporangial complex. (Fig. 53, bar = 40µm, all other figures, bar = 16 µm).

20-31 ($\bar{x}$ 24.4 ± 0.6) µm diam., with spines of upto 6 µm long and upto 3.5 µm in basal diameter. **Antheridia** absent. **Oospores** spherical, aplerotic, one per oogonium, 17-27 ($\bar{x}$ 22.6 ± 0.6) µm diam. with a wall approximately 2 µm thick.

Pythium oligandrum is abundant in the North of France. It was isolated from soil samples in the Compiègne as well as Lille regions. The above description is that of isolate F-81 isolated from Lille. There are some minor differences in the oogonial and oosporal dimensions of this isolate and those found in the literature, however all the other morphological characters of this fungus are identical. This is the first taxonomic treatment of *Pythium oligandrum* isolated in France.

***Pythium mamillatum* Meurs** (Figs 34-43).

Colonies on CMA & PCA produces some aaerial mycelium and shows a rosette pattern on the latter. Average daily growth on PCA at 25°C is 20 mm. Main hyphae are up to 7 µm wide. **Sporangia** or hyphal swellings are globose, ovoidal, ellipsoidal to somewhat cylindrical, intercalary, at times terminal, measuring 13 to 28 µm diam. ($\bar{x}$ 19.4 ± 0.6). **Oogonia** intercalary or terminal, globose to slightly ovoidal, provided with blunt to conical, and at times, curved spines 2-6 µm long and 1-3 µm broad. Oogonia 13-24 µm in diam. ($\bar{x}$ 18.3 ± 0.6). **Antheridia** mostly monoclinous rarely diclinous, usually one, infrequently two per oogonium, antheridial cells clavate making apical contact with the female gametangia. **Oospores** globose, plerotic, one per oogonium, measuring 12-22 ($\bar{x}$ 16.4 ± 0.6) µm diam., provided with a moderately thin wall of up to 2 µm.

The above description is of isolate no F-60, isolated from the region of Compiègne. The other two isolates of this species were collected in Lille. All the features of this fungus resembles to those found in the literature. The only difference is the absence of zoospores inspote of the presence of sporangia. The fungus failed to sporulate despite of repeated flooding in cultures with distilled water, soil extract water and maintenance at different temperatures. This is the first taxonomic description of *Pythium mamillatum* isolated in France.

***Pythium echinulatum* Matthews** (Figs 44-51 & Figs 56-57)

Colonies on CMA & PCA submerged showing an indistinct radiate pattern. Daily growth rate on PCA at 25°C 10 mm. Main hyphae upto 7-8 µm wide. **Sporangia** globose to cylindrical, terminal or intercalary, more often in chains, 11-25 µm diam. ($\bar{x}$ 17 ± 0.6). **Oogonia** terminal, intercalary, catenulate, globose to cylindrical, provided with acute conical spines 3-9 µm long. Oogonia 9-26 ($\bar{x}$ 18 ± 0.6) µm diam. (excluding spines). **Antheridia** mostly hypogynous at times mono and diclinous, 1-2 per oogonium. **Oospores** globose, rarely oval, plerotic or aplerotic, usually single, rarely two per oogonium, 6-24 ($\bar{x}$ 15 ± 0.6)µ diam. Wall 1.5-2 µm thick.

Pythium echinulatum was frequently isolated from the north of France. Nevertheless this species shows a great morphological variation. Acute conical spines on the oogonia, hypogynous antheridia, aplerotic and plerotic oospores were common in all the isolates, but the presence of catenulate sporangia and oogonia were not constantly found in all the isolates.

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POST-HARVEST ROTS OF TOMATO IN RELATION TO LYASES AND MYCOTOXIN PRODUCTION *IN VITRO* AND *IN VIVO*

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ABSTRACT - The post-harvest tomato rotting fungi were isolated from 100 samples of tomato showing rot symptoms. A total of 220 isolates were recovered on Czapek's dextrose agar medium comprising 6 fungal species among which *Alternaria alternata, Aspergillus flavus* and *A. niger* were predominant. *Asp. tamarii, Cochliobolus spicifer* and *Penicillium citrinum* were isolated at low frequency. Testing enzymatic abilities of some isolates showed that most of these isolates produced cellulase, pectin lyase and polygalacturonase on synthetic media ■ well ■ on inoculated tomatoes. Moreover, some isolates which did not show enzymatic activities on agar media produced enzymes on tomatoes. However, most isolates of *Alt. alternata, Asp. flavus* and *Asp. niger* tested were good enzyme producers and showed the highest enzymatic activity either *in vitro* or *in vivo* suggesting that tomato rot is mainly brought about by members of these fungi. It is worthmentioning that the ealthy fruits had no delectable enzymatic activity.

During screening for mycotoxigenicity, *Alternaria alternata* proved to be the most able mould to produce different mycotoxins in liquid medium as well as in infected tomatoes. In addition to *Alternaria* toxins, aflatoxins B_1, B_2 and citrinin were produced by *Asp. flavus* and *P. citrinum*, respectively. Results of this study clearly showed that tomato fruits infected naturally with the moulds previously mentioned may contain different mycotoxins which may represent a potential health hazard.

RESUMÉ - Les agents fongiques responsables du pourrissement après récolte des tomates ont été isolés de 100 tomates présentant des symptômes de pourrissement. Deux cent vingt souches ont été isolées sur Czapek-Dextrose agar. Ces souches se répartissent parmi six espèces. *Alternaria alternata, Aspergillus flavus* et *Aspergillus niger* sont les espèces les plus fréquentes. *Aspergillus tamarii, Cochliobolus spicifer* et *Penicillium citrinum* ont été isolés à de faibles fréquences. Des essais enzymatiques réalisés sur certains de ces isolats ont permis de montrer que la plupart produisent des cellulases, des pectine-lyases et des polygalacturonases, aussi bien sur milieu synthétique que sur tomates. Certains isolats ne présentent d'activité enzymatique que sur tomates. La plupart des isolats d'*Alt. alternata, Asp. flavus* et *Asp. niger* se sont révélés être de bons producteurs d'enzymes et montrent la plus haute activité, tant *in vitro* qu'*in vivo*, suggérant que le pourrissement des tomates puisse être incriminé à des membres de ces espèces. Les tomates saines ne présentent pas d'activité enzymatique.

Les études mycotoxicologiques ont montré qu'*Alt. alternata* était l'espèce isolée produisant le plus de mycotoxines différentes en milieu liquide ou sur tomates. En plus des toxines produites par *Alt. alternata*, des aflatoxines B1 et B2, et de la citrinine étaient produites respectivement par *Asp. flavus* et *Penicillium citrinum*. Il en résulte que des tomates naturellement contaminées pourraient contenir différentes mycotoxines, représentant un risque pour la santé.

INTRODUCTION

Tomato fruits represent one of the essential vegetables all over the world throughout the year. After harvesting, these fruits may be invaded by several moulds that probably cause fruit rotting and extensive damage to the crop (Ayres *et al.*, 1964; Barkai-Golan, 1974). Extensive deterioration results in economic loss to commercial marketers of these fruits. Tomato rot is favoured by the high temperature and hence it is pronounced in tropical and subtropical regions (Adisa, 1980) however, some fungi can infect tomatoes stored at 10-12°C causing their spoilage (Ayres *et al.*, 1964).

Infection of fruits by pathogenic fungi is initiated by production of cell wall-degrading and macerating enzymes (Weste, 1970). The role of polysaccharide degrading enzymes in microbial pathogenicity has been reviewed (Wood, 1976). The ability of many pathogenic moulds to produce these enzymes in culture is not sufficient reason to ascribe them a role in pathogenicity (Byrde, 1979).

In addition to biodeterioration of tomato fruits, different mycotoxins may be produced in these fruits by toxigenic moulds and this may constitute a potential health hazard (Harwig *et al.*, 1979; Stinson *et al.*, 1980 and 1981).

The present work was designed to throw some light on the moulds that cause post-harvest spoilage of tomato. Enzymatic abilities and mycotoxin-producing potential of these fungi, both *in vitro* and *in vivo*, were also studied.

MATERIALS AND METHODS

Source of samples. A total of 100 tomatoes showing lesions of different appearance were collected from markets in different localities of Assiut Governorate, Egypt.

Isolation and identification of moulds. By using sterile scalpal, tissue fragments were excised from lesions of infected fruits and were plated on Czapek's dextrose agar medium supplemented with rose bengal (65 ppm) as a bacteriostatic agent. Inoculated plates were incubated for 7-10 days at 28°C. The resulting moulds were isolated and identified.

Enzymatic activity of the isolated fungi. A total of 40 fungal isolates from fungi recovered during this investigation were screened for their ability to produce some enzymes on solid media as well as on tomato fruits. These fungi were *Alternaria alternata, Aspergillus flavus, A. niger, A. tamarii, Cochliobolus spicifer* and *Penicillium citrinum*.

Cellulase activity was studied by using the method described by Eggins & Pugh (1962). The tested fungi were grown on medium contained (g/L), ammonium sulphate, 0.5; L-asparagine, 0.5; potassium dihydrogen phosphate, 1.0; potassium chloride, 0.5; magnesium sulphate, 0.2; calcium chloride, 0.1; yeast extract, 0.5; cellulose, 10 and agar 20. After 7 days incubation at 28°C, plates were flooded with chloro-iodide of zinc. The uncoloured zone gave a measure of the cellulolytic power of the moulds.

The test isolates were screened on MP-7 and MP-5 media of Hankin *et al.* (1971) for pectin lyase (PL) and polygalacturonase (PG), respectively. After growth of organisms for 7 days at 28°C, pectolytic activities on both media were determined by flooding plates with 7 mol/L HCl solution. This precipitate intact pectin and pectolytic moulds were thus surrounded by clear zones against an opaque medium. The extent of zone of clearing around moulds was used as a measure of the degree of pectolytic activity.

Amylolytic activity of the test fungi were screened according to the methods described in the Society of American Bacteriologists (1957). The experimental medium consisted of 28 g of nutrient agar to which 2 g of soluble starch (Merck) were added per litre. After incubation of the inoculated plates for 7 days at 28°C in darkness, they were flooded with an iodine solution (KI, 15 g and I_2 , 3 g litre). A zone void of blue indicated the production of amylase.

Proteolytic activity of moulds was determined using a casein substrate. Each mould culture was inoculated onto the surface of mycological agar (peptone, 10 g; agar, 20 g per litre) to which sterile skim milk (10% solution of powder of defatted milk in water) was added at the rate of 5 ml per 100 ml of medium. After incubation for 7 days at 28°C, complete degradation of milk protein was seen as clearing zone in the somewhat opaque agar around colonies. The extent of the clear zone represented the degree of proteolytic activity.

Enzymatic activities of the tested fungi on tomatoes. Each tested mould was inoculated on the surface of tomatoes (individual weight, w = 70-100 g) disinfected with ethanol (90%). The inoculation was done by placing a square block of Czapek's dextrose agar with the fungal spores in two windowshaped wounds per fruit (Vinas *et al.*, 1992). Inoculated fruits were placed in sterile plastic bags and incubated at 28°C for 10 days. Two tomatoes were utilized for each strain.

After incubation period, the decayed tomatoes were taken, blended with 0.9 (v/w) distilled water for 3 min. Fruit extracts were clarified by centrifugation at 15000 x g for 15 min at 4°C. The supernatants were employed as crude enzyme solutions.

Plates containing different solid media specific for detection of amylase, protease, cellulase and pectinases were prepared as previously showed. Under sterilized conditions, 0.5 ml of the enzyme solution was pipetted in a cup made in the center of each plate. After incubation at 28°C for 24 h, the presence of these enzymes was examined.

Screening for mycotoxigenicity. Ten isolates of both *Alternaria alternata* and *Aspergillus flavus* and 5 isolates of each of *Asp. niger*, *Asp. tamarii* and *Penicillium citrinum* isolated during this study were screened for mycotoxigenicity on liquid medium as well as on tomatoes.

Inoculation and incubation procedures. The tested fungi were grown on yeast extract sucrose medium (YES). Spore suspension of a 7-days old culture of each mould was made and 0.5 ml (approx. 10^6 spores/ml) was used as an inoculum for each 50 ml quantity of YES medium in 250 ml Erlenmyer flasks. The flasks were incubated

at 28°C for 10 days as stationary cultures under darkness. Two replicates of each strain were analysed.

Like wise, each tested mould was inoculated on the surface of tomatoes. Inoculation and incubation procedures were previously mentioned. Tomatoes were frozen after they had reached the desired stage of rot, as estimated by the extent of external discoloration and kept untill extraction.

Extraction procedures. In case of liquid medium, the contents of each flask were homogenized with 50 ml chloroform for 5 min in a high speed blender (16000 rpm). Extraction was repeated three times. The combined chloroform extract was washed with distilled water, dried over anhydrous sodium sulphate, filtered and dried to near dryness on a rotary evaporator. The residue was diluted with chloroform to one ml. The chloroform solution was analysed for the presence of aflatoxins (B_1 ,B_2 , G_1 and G_2), patulin and citrinin using thin-layer chromatography.

In case of *Alternaria*, liquid cultures were extracted twice with ethyl acetate (30 ml) by overnight shaking under darkness and filtration. The two extracts were combined, dried over anhydrous sodium sulphate and evaporated to near dryness. The residue was dissolved in 1 ml of methylene chloride and analysed for the presence of alternariol (AOH), alternariol monomethyl ether (AME) and tenuazonic acid (TeA) on TLC.

The decayed frozen tomatoes were extracted according to Harwig *et al.* (1979). The fruits were thawed, blended with 0.9 (v/w) methanol and 50 ml of n-hexane for 3 min, and then centrifuged at about 2000 rpm for 5 min. for citrinin analysis (*P. citrinum*) , 3 ml of 10 N sulfuric acid was added to the extraction solvent. An aqueous methanol extract was removed by pipette and shaken with two 25-ml portions of chloroform. In case of *Alternaria alternata* analysed for AOH, AME and TeA, 3 ml of 10 N sulfuric acid was added to the aqueous methanol portion. Chloroform extracts were combined and evaporated to near dryness. The residue was dissolved in 1 ml of chloroform and was analysed for the presence of toxins previously mentioned.

TLC analysis. This was performed on precoated silica gel plates of kieselegel G type 60 (MERCK) of about 0.3 mm thickness using chloroform-acetone (9-1) as a solvent system. The developed plates were examined under UV light at wave lengths of 254 and 366 nm. Mycotoxins were identified by comparison with appropriate reference standards before and after treatment with p-anisaldehyde and ferric chloride solution as described by Durackova *et al.* (1976). *Alternaria* toxins were analysed according to Harwig *et al.* (1979). Authentic samples of aflatoxins and *Alternaria* toxins were purchased from Sigma Chemical CO., U.S.A. Patulin and Citrinin were obtained from U.S. Department of Agriculture, Northern Research Laboratories, Peoria, Illinois, U.S.A. ,

RESULTS AND DISCUSSION

During this study, *Alternaria alternata, Aspergillus flavus, Asp. niger, Asp. tamarii, Cochliobolus spicifer* and *Penicillium citrinum* were isolated from deteriorated

tomato fruits. As indicated in Table I, *Alt. alternata* was isolated from 70% of samples followed by *Asp. flavus* (62%) and *Asp. niger* (54%) suggesting the responsibility of these three moulds for biodeterioration of tomatoes. These results confirmed with those of Adebanjo & Shopeju (1993) who isolated *Asp. flavus* and *Asp. niger* at high frequencies from some sundried vegetables over a period of 8 weeks. Also, *Asp. flavus* was reported to be associated with the spoilage of tomato fruits (Fajola, 1979). In a similar study, *Alternaria* spp. were isolated from as many as 51.6% of decaying tomatoes stored at 10-12°C (Ayres *et al.*, 1964). The involvement of *Alt. alternata* in spoilage of stored tomatoes was reported by other workers (Pearson & Hall, 1975). ,

Results presented in Table II revealed that most strains of isolated fungi exhibited cellulolytic, pectolytic, amylolytic and proteolytic abilities when grown on

Table I: Fungi isolated from tomato fruit infected by rotting fungi.

Fungi isolated	Number of cases of isolation (out of 100 samples)
Alternaria alternata	70
Aspergillus flavus	62
Asp. niger	50
Asp. tamarii	15
Cochliobolus spicifer	8
Penicillium citrinum	15

Table II: Enzymatic abilities of some moulds, isolated from deteriorated tomatoes, in agar media.

Organism	Number of tested isolates	Cellulase	Pectin lyase	Polygalac-turonase	Amylase	Protease
Alt. alternata	10	5+++ 3++ 2-ve	10++	6+++ 2++ 2-ve	5++ 2+ 3-ve	6++ 2+ 2-ve
Asp. flavus	10	8+++ 2-ve	8+++ 2-ve	5+++ 3++ 2+	3+++ 3++ 2+ 2-ve	5++ 2+ 3-ve
Asp. niger	5	5++	5++	3+++ 2-ve	4++ 1-ve	3++ 2-ve
Asp. tamarii	5	3++ 2+	4+ 1-ve	3++ 1+ 1-ve	1++ 3+ 1-ve	3+ 2-ve
C. spicifer	5	5++	3+ 2-ve	4+ 1-ve	3+ 2-ve	2++ 2+ 1-ve
P. citrinum	5	2++ 3+	5+	3+ 2-ve	2++ 2+ 1-ve	2++ 3+

+, low activity; +++, high activity; ++, fair activity; -ve, no activity.

synthetic media. On inoculated fruits (Table III), the production of cellulase and pectinases was more pronounced. On the other hand, protease and amylase production was varied since some tested isolates which produced these enzymes on synthetic media did not exihibt any enzymatic activity on tomatoes. In this respect, Adisa (1985) found that cellulase, polymethylgalacturonase and pectinmethyltrans-eliminase were identified *in vivo* and in culture filtrates of two tomato spoilage moulds, *(Asp. flavus* and *Asp. fumigatus*. It was realised that the softening of tissues in ripening peaches was correlated with increase in the pectinic acid content (Shewflet *et al.*, 1971) and increased pectic enzyme activity (Pressey & Avants, 1971). These enzymes bring about the breakdown of polysaccharide components resulting in maceration of tissue and death of host cells. Among cell wall-degrading enzymes, pectinolytic and cellulolytic enzymes

Table III: Enzymatic abilities of some moulds, isolated from deteriorated tomatoes, in inoculated fruits.

Organism	Number of tested isolates	Cellulase	Pectin lyase	Polygalac-turonase	Amylase	Protease
Alt. alternata	10	10+++	6++ 4+	7+++ 3+	4+ 6-ve	5+ 5-ve
Asp. flavus	10	9++ 1-ve	8++ 2+	6++ 4+	3+ 7-ve	6+ 4-ve
Asp. niger	5	5+	5+	4++ 1-ve	2+ 3-ve	3+ 2-ve
Asp. tamarii	5	3++ 1+ 1-ve	4+ 1-ve	4+ 1-ve	3+ 2-ve	3+ 2-ve
C. spicifer	5	5+	4+ 1-ve	3+ 2-ve	2+ 3-ve	2+ 3-ve
P. citrinum	5	5+	5+	5+	4+ 1-ve	4+ 1ve

+, low activity; +++, high activity; ++, fair activity; -ve, no activity.

pose a unique position and several references referred the pathogenicity of plant pathogens to the ability of secretion of these enzymes (Weste, 1970; Kachhawaha & Ali, 1982), in spite of the involvement of other enzymes in cell wall degradation. This is because cellulose and pectin represent the main and most complex components of plant cell wall.

During screening for mycotoxigenicity of the tested fungi, 14 out of 35 isolates were toxigenic (Table IV). The toxigenic isolates belonged to *Alternaria alternata* (8 isolates), *A. flavus* and *P. citrinum* (3 isolates for each).

Alternaria alternata proved to be the most able species in mycotoxin production where 80% of its tested isolates produced toxins both *in vitro* and *in vivo*. Four isolates produced tenuazonic acid (TeA), 2 isolates produced tenuazonic acid (TeA) in addition to alternariol monomethyl ether (AME) and 2 isolates produced alternariol monomethyl ether (AME) as well as alternariol (AOH). These results suggest that tomato fruits infected naturally with *Alternaria alternata* may contain TeA,

AME and AOH. These compounds are known metabolites of *Alternaria alternata* (Pero *et al.*, 1973).

These results agree with those of Stinson *et al.* (1980) who found that most isolates of *Alternaria* produced TeA, AOH and AME on tomato fruits. They also stated that the known toxigenic strains of *Alternaria* produced more of TeA on tomatoes than the dibenzo-7α-pyrone toxins (AOH and AME). In another study, Stinson *et al.* (1981) reported that TeA was the main mycotoxin produced in *Alternaria*-infected tomatoes from commercial sources while AOH and AME were present in small amounts. Similarly, Harwig *et al.* (1979) found that *Alternaria alternata*, isolated from decayed tomatoes, produced TeA and AME in culture medium as well as in infected tomatoes however, AOH was not detected.

Table IV: Ability of some moulds, isolated from deteriorated tomatoes, to produce mycotoxins in liquid medium and in infected fruits.

Organism	Number of tested isolates	On liquid medium		On tomatoes	
		Number of toxigenic isolates	Mycotoxins detected	Number of toxigenic isolates	Mycotoxins detected
Alternaria alternata	10	[illegible]	Alternarial (AOH), Alternariol, Monomethyl ether (AME) & Tenuazonic acid (TeA)	[illegible]	Alternarial (AOH), Alternariol, Monomethyl ether (AME) & Tenuazonic acid (TeA)
Aspergillus flavus	10	3	Aflatoxins B1 & B2	3	Aflatoxins B1 & B2
Asp. niger	5	-	-	-	-
Asp. tamarii	5	-	-	-	-
Penicillium citrinum	5	3	Citrinin	3	Citrinin

From 10 isolates of *Asp. flavus*, one isolate produced aflatoxin B and 2 isolates produced aflatoxins B_1 and B_2. Published literatures dealing with the production of aflatoxins in infected tomatoes are not available. However, the natural occurrence of aflatoxins in tomato paste samples has been recorded (Saber *et al.*, 1992). In a similar study, Neelakantan *et al.* 1983) found that aflatoxin B was naturally present in apples. Experimental production of aflatoxins in various fruits has been reported (Detroy *et al.*, 1971).

Citrinin was produced by 3 isolates (60%) of *P.citrinum* in YES medium and in tomato fruits. These results agree, to some extent, with those of Harwig *et al.* (1979) who recorded this toxin in culture filtrate as well as in tomatoes inoculated with *P. expansum*.

Results of the present study clearly showed that most of the moulds that cause post-harvest spoilage of tomatoes are enzymatically active. These moulds excrete an array of enzymes which bring about the breakdown of organic matter resulting in

maceration of fruit tissues. In addition to biodeterioration, different mycotoxins may be produced in fruits by toxigenic moulds which represents a potential health hazard.

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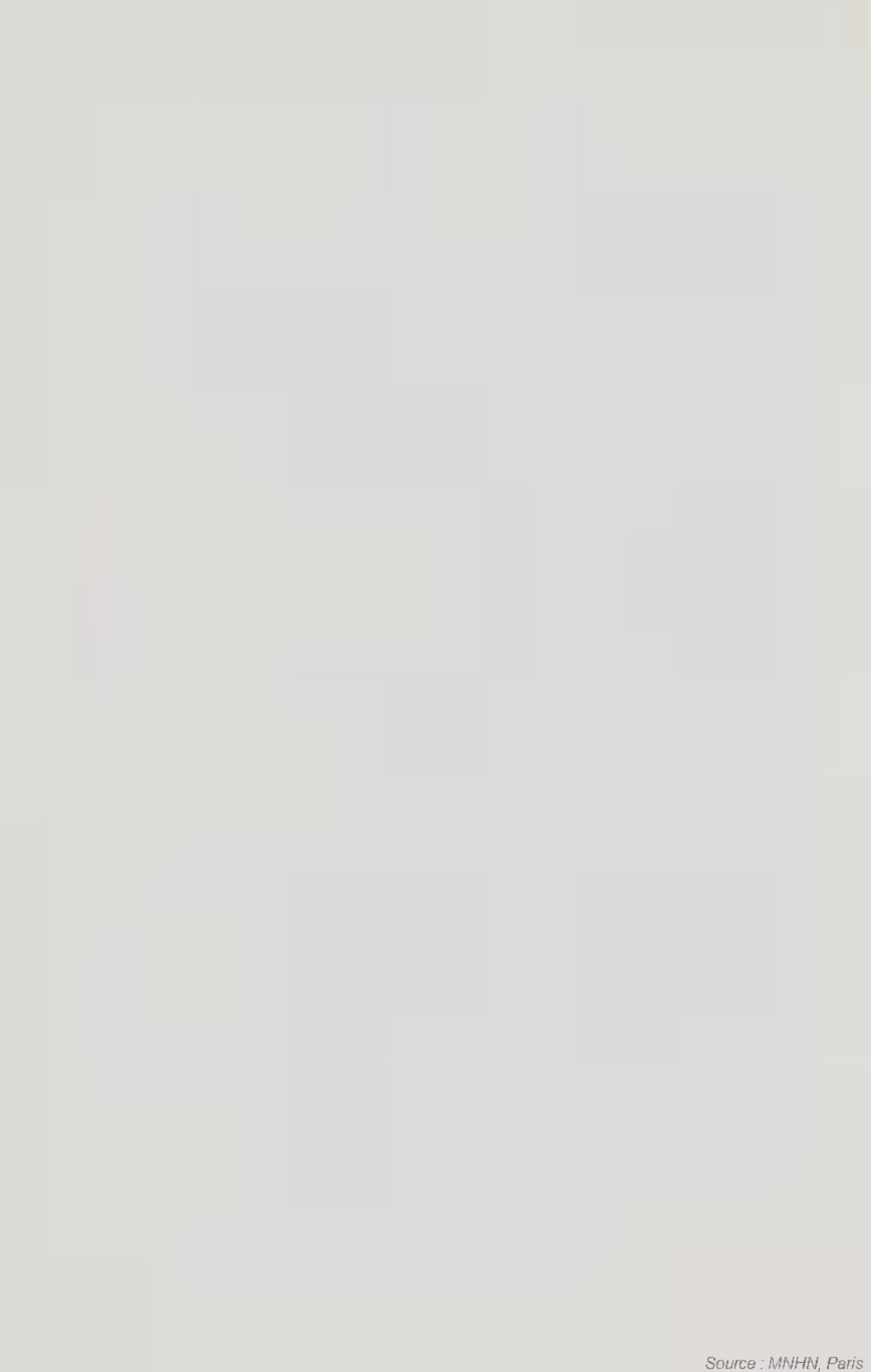

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ANALYSES BIBLIOGRAPHIQUES

REED C.F. & D.F. FARR : **Index to Saccardo's Sylloge Fungorum** Volumes I-XXVI in XXIX, 1882-1972. - Contribution N° XXXI of the Reed Library and Herbarium, Darlington, Maryland; Contribution N° 6 from the U. S. National Fungus Collection, Beltsville, Maryland; Rose Printing Company, Tallahasseee, Florida. Hardback $ 75, Softback $ 60, plus $ 4 for shipping. Obtainable from Clyde Reed, 1222 Main St., Darlington, MD 21034, USA.

Le Sylloge Fungorum est un répertoire des noms et des descriptions des espèces fongiques et bactériennes proposées entre la fin du XIXème et le début du XXème siècle. Les vingt-cinq volumes de cette oeuvre, presque encyclopédique, du célèbre mycologue italien P.A. SACCARDO et de ses collaborateurs, furent publiés entre 1882 et 1931; le volume n° 26 a été édité en 1972. Cette série demeure l'élément de référence de base en taxonomie de champignons. On y trouve également les auteurs des taxa proposés et les références bibliographiques majeures les concernant. Seul point négatif de cette compilation critique magistrale, l'absence d'un mode cohérent de présentation de l'énorme masse d'informations contenues dans ses divers volumes. Ce mode particulier de présentation est à l'origine des difficultés constantes, rencontrées lors d'une recherche des caractéristiques morphologiques d'un taxon donné; en particulier, si le nom recherché n'est pas répertorié dans le premier volume, puisqu'un index cumulatif n'était pas disponible, d'où l'impérieuse nécessité de préparer un index général couvrant la totalité du Sylloge.

L'Index du Sylloge Fungorum volume I - XXVI dans XXIX, 1881-1972, de Reed et Farr constitue ce document cumulatif, tant désiré, des nombreux volumes de cette oeuvre magistrale. Seul le titre n° 13 afférent aux noms des plantes-hôtes, n'a pas été inclus dans l'index réalisé par ces deux auteurs. Dans une courte introduction de trois pages, Reed et Farr passent rapidement en revue les problèmes rencontrés lors de la préparation de cet index: problèmes de pagination, certaines pages du Sylloge n'ayant jamais été numérotées; conformité de la pagination orignale avec celle de la première réédition du Sylloge réalisée par Edwards en 1944, etc... D'ailleurs, une copie des quatre pages manquantes dans l'édition d'Edwards a été ajoutée à la fin de cet Index; ceci permet aux détenteurs de copie de cette dernière de combler cette lacune.

La réalisation de cet Index ne pouvait être envisagée sans l'existence des moyens électroniques modernes de traitement de l'information. L'utilisation d'une banque de données informatique rend possible le traitement de la totalité des 121 627 noms figurant dans le Sylloge. L'informatisation des éléments taxonomiques de chaque binôme a permis, par ailleurs, la mise en évidence, de certaines corrélations intéressantes: répartition des noms recensés par volumes de citation, définition de la masse des genres traités et établissement des listes respectives des espèces afférentes, fréquences d'utilisation des épithètes spécifiques, mode de répartition de ces épithètes d'après la fréquence de la première lettre alphabétique employée, etc ... On y découvre ainsi que dix des genres traités comportent chacun plus de mille espèces, que l'épithète spécifique

elegans se distingue par une fréquence optimale d'utilisation (151), que la lettre c de l'alphabet est la première lettre de 12,5 % des noms d'espèces repertoriés alors que la lettre y ne concerne que 0,1% de ces noms, etc...

Après cette courte introduction, le Conspectus du Sylloge Fungurom reproduit, sur dix pages, les références bibliographiques de ses divers volumes et les sommaires respectifs; ces derniers comportent la liste des ordres, familles et sections traités dans chaque volume avec indication des numéros des pages afférentes. Cette synthèse permet une vue générale des groupes taxonomiques traités, rangés d'après le système particulier mis au point par Saccardo lui-même.

Le coeur de l'ouvrage se compose de plus de huit cents pages de format A4; c'est l'Index des noms figurant dans le Sylloge Fungorum, repértoriés sur deux colonnes par page, un mode de présentation favorisant un balayage visuel rapide du contenu de chaque page. 4827 genres sont ainsi répértoriés par ordre alphabétique. Pour chaque genre cité est précisé le nom(s) d'auteur(s), les éléments de la référence bibliographique originale et des informations d'ordre supragénérique: ordre et famille d'appartenance. A l'intérieur des genres, les noms des espèces qui en relèvent sont également présentées en ordre alphabétique, accompagnés chacun des numéros de volumes et des pages de citations dans le Sylloge. Point important, les synonymes ont été également répértoriés avec indication entre parenthèses du nom correct admis dans le Sylloge.

Il ne fait aucun doute que cet index tant attendu devient un outil inestimable et indispensable pour les mycologues poursuivant des recherches en taxonomie des champignons; également pour tout chercheur amené à résoudre un problème d'ordre nomenclatural concernant ces microorganismes. Sa réalisation a exigé un effort colossal répartis sur plusieurs années et cela malgré le recours massif aux moyens modernes de traitement de texte; d'ailleurs sans l'avènement de l'informatique, cet index n'aurait sans conteste pas vu le jour. D'autres mycologues ont également contribué, de manière bénévole, à la solution des problèmes soulevés par cet essai d'intégration en une masse cohérente du contenu des volumes du Sylloge. Les descriptions originales des espèces répertoriées ainsi que les références afférentes peuvent maintenant être rapidement localisées en un minimum de temps, sans recherches bibliographiques vaines à travers le dédale des volumes du Sylloge. Plus important encore, ce nouvel index permet aux personnes ne possédant pas une copie originale ou une reproduction du Sylloge, de démarrer une recherche bibliographique efficace et rapide pour un genre déterminé.

Il reste enfin à signaler qu'une liste des noms omis dans le Sylloge a été antérieurement préparée par P. M. KIRK; cette liste fut publiée en 1985 par le Commonwealth Mycological Institute.

Jean MOUCHACCA

ARORA D.K., ELANDER R.P. & MUKERJI K.G. (Eds) : **Handbook of Applied Mycology.** Vol. 4 - Fungal Biotechnology, Marcel Dekker Inc., 270 Madison Ave., New York, NY 10016, 1992. 1114 pp. Price £ 150.00; by subscription $ 127.00.

La mycologie appliquée est actuellement devenue une discipline biologique comparativement très stimulante et un champ d'action en évolution rapide. Cette acti-

vité scientifique progesse grâce à une intégration harmonieuse d'un ensemble de spécialités relevant de disciplines variées: agricoles, industrielles, pharmocologiques, médicales et alimentaires. Cette définition de la mycologie appliquée est proposée par D. K. ARORA, principal éditeur de la série des cinq manuels des "Handbook of Applied Mycology". L'ouvrage "Fungal Biotechnology" en est le quatrième volume. Ce livre de dimensions marquées, comporte quatre sections de volume inégal et plus de mille pages de texte. Les trois volumes antérieurs ont porté sur la nature des liens entre les Sols et les Plantes, entre l'Homme, les Animaux et les Insectes et, enfin, sur ceux impliquant les Champignons dans l'Alimentation Humaine et Animale.

Selon les trois éditeurs du quatrième volume, la biotechnologie des champignons est l'utilisation des organismes fongiques ou de leurs composants subcellulaires, dans des processus technologiques appliqués dans des domaines de productions industrielles ou de gestion de l'environnement. Cette discipline requiert des connaissances approfondies en génétique, biologie moléculaire et biochime des champignons et également en chimie analytique ou autre. L'ouvrage traite successivement des thèmes majeurs suivants : Technologies moléculaires, Applications commerciales, Décomposition des résidus biologiques et chimiques et Collections de culture, aspects légaux et sécurité biologique.

Cet ouvrage bénéficie d'un chapitre introductif traitant de la biotechnologie des champignons, une synthèse liminaire qui constitut la première contribution de la section Technologies moléculaires. Ses deux auteurs Elander & Lowe passent en revue les processus fongiques commercialiés à ce jour et qui, globalement, font appel à des espèces fongiques, toutes capables d'un bon développement sur des milieux de culture à coûts de production excessivement faibles. Ce chapitre est suivi par une analyse judicieuse des approches molécularistes en taxonomie des champignons: description des stratégies développées et avantages et inconvenients des techniques analytiques mises au point: ses deux auteurs, Klich et Mullaney, insistent sur le fait que la biologie moléculaire n'offre pas de solutions miracles pour les controverses ou disputes d'école existant en taxonomie des champignons. Cette première section comporte dix chapitres avec des contributions marquées sur la technologie des protoplastes, sur les plasmides fongiques et la transformation et manipulations génétiques chez les champignons filamenteux.

La deuxième section rassemble une série de contributions sans lien apparent. On y trouve deux articles sur les champignons thermophiles: rôle en agriculture et potentiel biotechnologique et un autre sur les mycorhizes; ce dernier sujet a été largement débattu dans le premier volume de cette série. La troisième section (quatre chapitres), comporte des contributions bien structurées à l'échelle individuelle mais sans aucune trame collective convaincante; le thème global traité aurait pu d'ailleurs faire l'objet d'un volume distinct. Enfin, la dernière section relative aux collections de cultures, propose trois contributions analysant les problèmes liés à l'établissement des brevets et la conclusion des accords commerciaux secrets, seule forme légale de protection dans le cas des inventions biotechnologiques.

Globalement et malgré une certaine dose de répétition, le quatrième volume de cette série propose une masse importante d'informations sur les sujets abordés, surtout, dans certains cas, au niveau des références bibliographiques afférentes. Les trente-quatre contributions proposées, quoique de poids relatif inégal, constituent néanmoins d'excellents articles de synthèse. Ceux-ci seront largement appréciés par les chercheurs débutants ou confirmés désireux de parfaire leur connaissance sur un sujet approprié ou d'acquérir des notions introductives sur un thème inédit. Il reste à souligner que le faible prix demandé pour cet ouvrage est de nature à favoriser son achat par les bibliothèques institutionnelles.

Jean MOUCHACCA

BHATNAGAR D., LILLEHOY E. B. & ARORA D. K. (Eds) : **Handbook of Applied Mycology.** Vol. 5 - Mycotoxins in Ecological Systems, Marcel Dekker Inc., 270 Madison Ave., New York, NY 10016, 1992. 443 pp. Price £ 150.00; by subscription $ 127.00. prix à voir

Ce dernier volume de la série des manuels à multi-auteurs de mycologie appliquée a pour titre: les mycotoxicoses dans les systèmes écologiques. Dans la préface, les éditeurs soulignent qu'aprés l'épopée héroïque des antibiotiques d'origine fongique, les recherches en mycotoxines se sont rapidement développées pour devenir des thèmes majeurs en mycologie appliquée. En efffet, la mycotoxicologie a définitivement acquis ses lettres de noblesse; elle n'en demeure pas moins une activité scientifique à caractère multidisciplinaire. Ce champ d'action soulève cependant une question fondamentale qui reste encore un sujet de controverses : Quel est le fondement mystérieux de la logique biologique conduisant à la production de métabolites secondaires par les champignons ? Ces composés chimiques sont à l'origine des symptômes toxicologiques enregistrés.

Cet ouvrage de quatre cents pages comporte seize contributions, non rassemblées en sections autour de thèmes particuliers moins généraux. En parcourant les titres proposés, il devient évident que le contenu de certains articles s'éloigne quelque peu du titre général de l'ouvrage. Ces chapitres revêtent un caractère purement descriptif du sujet traité sans qu'un système écologique donné y soit incriminé. Ceci dit la qualité de l'ensemble des textes retenus reste de haut niveau: ce sont des synthèses actualisées de grande importance.

Le fil conducteur de quelques chapitres semble être les effets intracellulaires des mycotoxines, actives dans certains systèmes animaux. En particulier, ces chapitres traitent des mécanismes de cytotoxicité et de genotoxicité attribués aux aflatoxines. D'autre part, les mécanismes subcellulaires de toxicité, attribués à l'acide cyclopiazonic et à l'ochratoxine, font également l'objet de deux chapitres intéressants. La biosynthèse et la régulation de la production des aflatoxines et des trichothécenes sont également traitées de manière exhaustive dans diverses contributions; celles-ci n'intègrent pas

malheureusement et de manière détaillée, des corrélations écologiques afférentes aux sujets abordés.

On peut regretter que le panel des rédacteurs sollicités rassemble presque exclusivement des spécialistes travaillant dans des institutions nord-américaines et européennes, où se réalise en réalité la quasi-totalité des recherches consacrées aux problèmes de toxicologie d'origine fongique. La parution de cet ouvrage sera, néanmoins, largement acceuillie à l'échelle internationale, par tous les chercheurs spécialisés ou non spécialisés, intéressés par les déréglements métaboliques résultant du développement végétatif des champignons filamenteux sur des productions industrielles animales et agricoles. En effet, les mises à jour proposées permettent de très rapidement acquérir une vue d'ensemble approfondie d'un sujet déterminé, et surtout, de dégager des futurs points de recherches dans des thèmes en cours d'investigation.

La bibliograhie afférente à chaque article est abondante et comporte une bonne proportion de titres récents. Le dernier volume de cette série devrait rapidement trouver sa place dans les bibliothèques des laboratoires intéressés. Il sera consulté par un large éventail de spécialistes travaillant dans des domaines diversifiés et touchant, de prés ou de loin, à la production, la distribution, la commercialisation et la consommation de productions agricoles et animales industrielles.

Jean MOUCHACCA

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TABLE DU TOME 15

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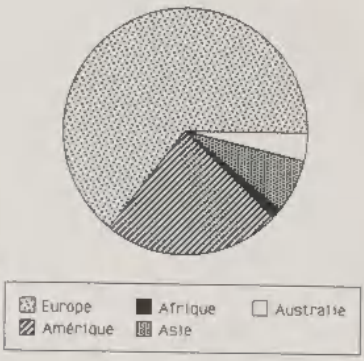

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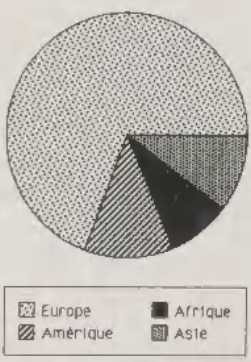

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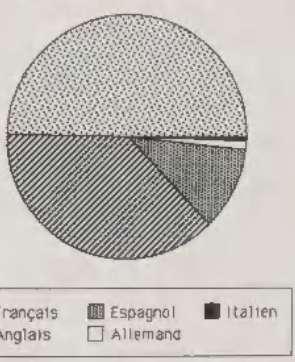

SOMMAIRE

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